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STUDIES ON THE EMBRYOGENY AND POSTNATAL DEVELOPMENT OF THE APHIDIDÆ WITH SPECIAL REFERENCE TO THE HISTORY OF THE "SYMBIOTIC ORGAN," OR "MYCETOM" [1]

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THIRTEEN PLATES

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[1] Contribution from the entomological laboratories of the Bussey Institution, Harvard University, No. 202. Received for publication May 17, 1922. The Bussey Institution and the University of the Philippines have contributed funds covering a large part of the cost of making the halftone plates for this paper.

INTRODUCTION

The Aphididæ have attracted the attention of naturalists for a long time, on account of their economic importance as pests of many cultivated plants, their peculiar method of reproduction, and other interesting habits. It is unfortunate however that, as Baker (1920) has recently pointed out, taxonomic studies on this family have not kept pace with the anatomical and the biologic, with the result that many of the earlier investigators have used species of doubtful identity, which fact makes adequate comparison and correlation of results obtained by different workers impracticable in a number of cases. This difficult condition had been noted years ago by Stevens (1906). It is encouraging to note however that, since her time, a few very able and enthusiastic systematists, principally in America and in Europe, have interested themselves in working out a more comprehensive classification of aphids.

In the field of aphid embryology, the more important papers bearing on the subject, particularly on the parthenogenetic form, are products of the past century. These publications are in general models of care and skill in preparation. They have furnished an important and very useful working basis for subsequent investigators.

The present work is an attempt to gather additional data on the embryonic and postembryonic development of aphids. The investigation has been almost exclusively confined to parthenogenetic individuals; but I am hoping to be able to make a more extensive study of the amphigonous material in the future, as time and opportunity permit. Particular effort is made in the present work to study the ontogenetic cycle of the peculiar granular inclusions in certain specialized cells, which are known as mycetocytes. These granular bodies are believed to be micro-

organisms of a symbiotic nature. The organs harboring these supposed microörganisms, which have been given the name mycetom (Šulc, 1910), have a very interesting history. An attempt is made in the present work to follow their early formation and subsequent development.

Preliminary work was done on the problem in the fall of 1919, at the suggestion of Prof. W. M. Wheeler, under whose direction the present investigation was carried on. I take this opportunity to express to him my thanks for encouragement and helpful advice during the progress of the work, and also for generous loan of important pamphlets from his private library. Grateful acknowledgment is likewise due to Prof. C. T. Brues and to other members of the staff of the Bussey Institution, upon whom I have called for assistance, both scientific and technical, from time to time.

HISTORICAL

The literature bearing on aphid reproduction, embryology, parthenogenesis, and symbiosis is very extensive. Some very careful historical discussion along these lines has been published from time to time; but there appears to be some justification for preparing another review in that the previous ones only partially cover their respective fields. Further, these older treatises are scattered in different publications, some of which are not easily accessible. In the preparation of the present review, the papers of the following authors have been frequently consulted: Buckton (1876); Taschenberg (1892); Phillips (1903); Berlese (1909, pp. 5–29); Šulc (1910); and Buchner (1912, 1921).

REVIEW OF THE LITERATURE ON REPRODUCTION AND PARTHENOGENETIC EMBRYOLOGY IN APHIDS

The first observer to publish on aphids appears to be Leeuwenhoek (1695). In his Epistola 90 he records the viviparous production of several species of aphids, principally the one on *Ribes* sp. His inability to discover any male led him to believe that the viviparous young are produced by hermaphroditic individuals. About fifty years later, Réaumur (1737) noted the same method of unisexual reproduction. He was so blinded, however, by his belief that the union of biparental elements is necessary as a prerequisite to development of the embryo, that he insisted that the males had to be present, in spite of the fact that he himself had failed to discover any, and that copulation had had to take place.

It was not until Bonnet (1745), then a twenty-year-old youth, a student of Réaumur, undertook to raise nine consecutive viviparous generations in two and one-half months that it was proved, for the first time, that the male is dispensed with in this method of reproduction. The same investigator further observed, also for the first time, that in the case of oak aphids males are present and are in copula with the females, the latter, however, laying eggs instead of bearing their young "alive." He, nevertheless, failed either to grasp the significance of this occurrence of amphigonous generation or to notice that this oviparous form is an entirely distinct individual from the viviparous.

To de Geer (1771) belongs the credit of being the first to discover that an oviparous individual is always oviparous, and a viviparous always viviparous. His observations on *"Lachnus pini"* [2] and on *"Aphis rosæ"* led him to the conclusions that, of these species of aphids, viviparous individuals are found during the summer and that oviparous forms are present only in the fall. He ventured further to predict, on the basis of his studies, that, if aphids were to be discovered in the Tropics (practically nothing was known at that time about tropical aphids), they would be exclusively viviparous. He did not, however, note the relationship of the oviparous and the viviparous generations in that they alternate with each other in the yearly cycle of generations.

The first extensive experiments on viviparous reproduction were performed by Kyber (1815). He succeeded in raising for a period of four years consecutive viviparous generations of *"Aphis rosæ."* As I have noted in an earlier paper (Uichanco, 1921), it is quite probable that Kyber secured his experimental material from among a strain that normally reproduces exclusively by parthenogenesis.

Duvau (1825) succeeded in raising eleven continuous viviparous generations for a period of seven months, at the end of which time he noted that oviparous generation occurs. He was thus enabled to prove experimentally, for the first time, that the viviparous and the oviparous individuals are entirely distinct and irreversible forms, and that one of these generations in turn gives rise to the other. His views were confirmed by Morren (1836), who further advanced the theory that the young vivip-

[2] Unverified or unverifiable determinations are reported in the present paper in quotation marks.

arously produced aphid develops in the body of the parent by individualization of a previously organized tissue; by Ratzeburg (1844); and by von Heyden (1857), who claimed to have seen oviparous females actually copulate with males.

Duvau's conclusions were further strengthened by the findings of von Siebold (1839), who discovered for the first time in connection with aphids that oviparous individuals of *"Aphis loniceræ"* have a seminal receptacle, while the viviparous ones are devoid of this organ. He also noted that there is a difference in the early developmental history between a viviparous and an oviparous generation.

The views of Duvau were elaborated later by Steenstrup (1842), when the latter introduced the idea of alternation of generations in aphid reproduction. Steenstrup considered the viviparous mother not as female, but as nurse which is devoid of ovaries but which possesses a well-developed uterus. The viviparous young, according to him, arises through a process of budding.

Steenstrup's view found a supporter in the United States in Burnett (1853), who claimed that reproduction in viviparous aphids is a case of gemmiparity. He also failed to discover in these forms true ovaries.

Leydig (1848) arrived at entirely different conclusions. He pointed out that the viviparous embryos, like the oviparous, arise from eggs and are not produced by budding. He further noted that there was no observable difference between the viviparous and the oviparous eggs, except in the relative amount of yolk. In a later publication (Leydig, 1850), he reiterated his former views, and disproved Carus's (1849, p. 20) contention, published the year before, that the young of viviparous aphids arise from an amorphous central mass, in contradistinction to the cellular nature of the egg of oviparous individuals, the former being analogous to the "Keimschlauchen" of certain trematodes. It may be noted in this connection that in his last-cited paper, Leydig (1850) states that he saw the organs that we now know as "pseudovitellus," "mycetom," "green body," and "symbiotic organs," of which he wrote a recognizable description.

The questions as to whether or not the viviparous individuals are true females and the viviparously produced embryos arise from true eggs remained unsettled for many years. Even in the present day we still find a few authors who, influenced perhaps by the earlier views, have referred to this method of reproduction as "asexual." Von Siebold (1856) and Leuckart (1858)

were among the best known of the earlier supporters of the asexuality of the viviparous aphids, as previously announced by Steenstrup and by Carus. Huxley (1858) and Lubbock (1859) were somewhat noncommittal in their statements in regard to this point, but they, too, were obviously influenced by the old idea that fertilization is the only criterion of sexual reproduction so that they named the eggs formed in the ovaries of viviparous mothers "pseudova," as distinguished from the ova of the oviparous individuals, which they seemed to think were the only true ova. Among the opponents of such views during those times were Filippi (1856, p. 44) and Claus (1858), both of whom maintained that the viviparous forms are true females.

To Huxley (1858) belongs the credit of being the first to recognize the fact that viviparous reproduction in aphids is a true case of parthenogenesis, in the sense in which we use the word to-day.

Owen (1849) had previously used the same term, meaning however an alternation of generations, according to the idea of Steenstrup, the former further elaborating the latter's views in suggesting that copulation which takes place in the fall furnishes the "fertilizing influence," or "spermatic force," sufficient to bring about the development of the succeeding spring and summer generations. From his description (pp. 69 and 70) it appears that he had reference to the "symbiotic organs" when he spoke of "spermatic force."

Claus (1864) seconded Huxley's contention by stating that "die Keime der viviparen Blattläuse sind Eier, die sich parthenogenetisch entwickeln." The next important step in the advancement of our knowledge of parthenogenetic reproduction in aphids is the discovery by Blochmann (1887) that, while the amphigonous ovum extrudes two polar bodies, the parthenogenetic produces only one.

The later development of the parthenogenetic embryo of aphids was apparently first studied extensively by Huxley (1858), and reported in the paper which I have just cited.

In the same year Leuckart (1858), in a general treatise on insect reproduction, noted the fact that he could find no fundamental difference between the ontogeny of the parthenogenetic embryo and that of the amphigonous embryo, except that the former develops very early within the body of the mother.

A year later Lubbock (1859) published his paper on the ova and pseudova of insects, in which the part dealing with aphid reproduction is merely confirmatory of Huxley's observations.

The next important paper was that of Metschnikow [3] (1866), who took up the embryology of parthenogenetic aphids in much greater detail than any of his predecessors. Considering that at the time this paper was published Metschnikow was only twenty-one years of age, one cannot but admire the remarkable thoroughness with which this brilliant Russian genius attempted to treat a subject that in those days had been very little investigated.

Brandt (1869) noted for the first time the phenomenon of rotation (*Umklappung*) of the aphid embryo, as well as that of certain Heteroptera and Libellulidæ, thus antedating by many years the observations which Hallez (1886) subsequently formulated into a *"loi de l'orientation de l'embryon chez les insectes,"* now bearing the latter's name.

In the same year Balbiani (1869, 1870, 1870a, 1871, 1871a) began the publication, on instalments, of his *Mémoire,* which contains many interesting views on aphid embryology. This author was the first to work on the embryogeny of amphigonous aphids.

Brass (1883), Will (1883, 1889), and Witlaczil (1884) complete the list of the more important workers on aphid embryogeny during the past century. In his later paper Will had the advantage over his predecessors in that he was able to study aphid embryos for the first time with the aid of paraffine sections.

Of the more recent workers, it is by no means an easy task to give an adequate and unbiased account; a few of the more important contributors were Stevens (1905, 1906) and Tannreuther (1907), both of whom worked principally on the germ cells and early embryogeny, Hirschler (1912), de Baehr (1920, and also earlier papers), and others.

During the past few years, very creditable embryological papers on Coccidæ and on other Homoptera have also appeared.

REVIEW OF THE LITERATURE ON "SYMBIOSIS" AND ON "SYMBIOTIC ORGANS" IN APHIDS

As I have noted above, Leydig (1850) was the first investigator to publish a description of the symbiotic organs in aphids.

[3] Also spelled Mecznikow, Metschnikoff, Mečnikov, Metchnikoff, and possibly other variations. These different forms are apparently attempts at rendering into their approximate phonetic equivalents in Roman letters the original Russian characters. The spelling given in the text is adopted in the present paper, being the one used in his article which I cited.

He observed the organ early in the formation of the blastoderm in the parthenogenetic egg as an immigrating

> grüne oder gelbe körnige Masse . . . . . welche anfangs, wie es scheint, frei zwischen den Zellen liegt, später aber deutlich zu grösseren Ballen zusammengehäuft, von einer Membran umschlossen ist und sich an der Bildung der vegetativen Organe der Blattlaus beteiligt.

He did not give the organ any name; although, judging from his discussion, he apparently ascribed to it a nutritive function.

Huxley (1858) studied the formation of this organ which, he noted, appears as a central mass in the parthenogenetic egg; but he apparently overlooked its presence in the amphigonous form. He named the organ, as it occurs in the parthenogenetic ova, "pseudovitellus," since, according to him, "it completely simulates the vitellus of an impregnated [amphigonous] ovum." Lubbock (1859) adopted Huxley's views and name for the organ.

Metschnikow (1866) worked more carefully on the histology and ontogeny of the organ in the viviparous embryo. In that paper, he suggested that the function of the organ is nutritive, calling it "secundäres Dotter," as distinguished from the true, or primary yolk. He noted that the "secundäres Dotter" appears very early in the embryonic history, being found almost simultaneously with the first formation of the anlagen of the genitalia. He gave an account of the origin of the "secundären Dotter" from a large, subglobular, green cell, which in his figures 13 and 17A, Plate 28, is shown as consisting of a large mass of green, coarsely granular cytoplasm and a distinct nucleus and nucleolus. He claimed that this green cell first makes its appearance at the posterior pole of the egg, during the blastodermic formation of the latter, in a swelling which becomes united with the follicular epithelium.

Balbiani (1869, 1870a, 1871) traced the origin of this organ, which he renamed "masse polaire," to a "cellule antipode." The latter probably corresponds to the green cell described by Metschnikow. Balbiani objected to the theory proposed by Huxley, Lubbock, and Metschnikow on the ground that an animal so highly specialized as the aphid would not have yolk persisting throughout its life. Such a view, according to him, was physiological heresy. He revived Leewenhoek's idea of hermaphroditism in aphids, in which he was a strong believer, and suggested that the "masse polaire" is of an androblastic nature. This hypothesis is strikingly suggestive of Owen's (1849) old view, which I have noted above. Balbiani (1869) was the first inves-

tigator to report on the presence of the "masse polaire" in the amphigonous generation.

Witlaczil (1882, 1884) traced the origin of the organ in question from the follicular epithelium. He claimed to have seen a direct ingrowth of the latter into the blastocœle, forming thus a club-shaped body which ultimately becomes incorporated as an integral part in the constitution of the embryo. He proposed in his earlier paper (1882) another theory in regard to its function—that it takes the place of the Malpighian tubules which, as previously noted by Schindler (1878), are apparently absent in aphids. However, he gave up this view in his later paper (1884), for then he characterized these organs as being of a doubtful nature. Witlaczil (1882) also noted the presence of *tunica propria,* which envelops the "symbiotic organs."

Will (1889) reaffirmed the old views of Huxley, Lubbock, and Metschnikow that the "secundäre Dotter" is of a nutritive function. He had a very interesting acount of the early history of the formation of this organ, and in many cases his findings have been confirmed by subsequent workers. He noted that the "secundäre Dotter" originate in a thickening of the follicular epithelium toward the posterior pole of the egg, the protoplasm of the epithelial cells in the meantime assuming a

feinkörnige Beschaffenheit * * * und verwandelt sich in eine Summe feiner Dotterkörnchen, bei deren Bildung die Kerne in Mitleidenschaft gezogen werden und der Atrophie anheimfallen.

He proceeds:

So findet man bald an der verdickten Stelle des Follikelepithels nur noch eine feinkörnige Dottermasse ohne jede Spur der früher vorhandenen Kerne. Höchstens sind von den letzteren zur Zeit der Dottereinwanderung nur noch spärliche Trümmer in Gestalt zerstreuter Chromatingranula nachzuweisen.

He further questioned the contention of his predecessors to the effect that the formation of the "secundäres Dotter" consists in immigration of certain cells into the egg; for he claimed that only "todte Nahrungsmasse" finds its way into the blastocœle. He did not, however, note the relation of the "vitellophags" with the formation of the "symbiotic organ." He believed that the former represent the entoderm of the aphid embryo, and enter in the formation of the midgut.

Henneguy (1904), with material which he mainly borrowed from an unpublished work of Balbiani, also described the origin of the "pseudovitellus" from the follicular epithelium, as "une celle pediculee epitheliale," which arises by budding.

Flögel (1905) published a carefully written, although somewhat superficial, description of the first appearance of the "pseudovitellus" in the amphigonous eggs of "*Aphis ribis* Linnæus," the relation of this organ to the ovaries, and their subsequent history up to their bipartition as a result of the pressure exerted by the developing alimentary canal. He further noted that, after the birth of the young aphid, the cells of the "pseudovitellus" no longer divide and merely increase in size. Like some of his predecessors, Flögel ascribed to the "pseudovitellus" a nutritive function.

Stevens (1905), in casually mentioning the incursion of the "secondary yolk," spoke of "two conspicuous cells which apparently guard a valvular opening in the wall of the oviduct, and recall the four guard-cells at the inner end of the embryonic pharynx of *Planaria simplicissima*." She claimed that the "secondary yolk" material enters through this valve.

Tannreuther (1907) reported his inability to find such a valve in the aphids with which he worked. The same investigator claims to have noted the formation of "pseudovitellus" in parthenogenetic eggs, prior to cleavage of the latter, through immigration of certain cells from the follicular epithelium.

Šulc (1910) also reported having observed the presence of the "symbionts" between the egg and the follicular epithelium prior to maturation of the former, inferring therefrom that infection of the egg occurs before cleavage.

A somewhat similar view was held by Hirschler (1912), although his contention was apparently based on no direct evidence of his own.

Buchner (1912, p. 42) published some very interesting accounts of the infection of the winter eggs of aphids by the "symbionts" through a rupture in the follicular epithelium. The "organisms" which have escaped from the "mycetocytes" of the mother enter the developing egg through the opening thus made. He reiterated these views in a later publication (Buchner, 1921). In this book (pp. 211–218) he also gave a unique description of the formation of the "mycetom" in parthenogenetic eggs, with which my findings disagree in a few important particulars. In his text figures 58 and 60, drawn from hitherto unpublished illustrations by one of his students, and in his discussion on pages 212 and 214, he traced the origin of the "mycetom" in the parthenogenetic egg of aphids to a layer of vacuolated cells, which separates from the blastoderm at the posterior pole of the egg, beneath a subglobular mass of cells which he interpreted as the

primitive germ cells. He caused this anlage of the "mycetom" eventually to merge with the adjoining portion of the follicular epithelium. In the meantime, according to him, a "mycetom" cell in the cœlomic cavity of the mother forms a connection, through a special process in the epithelium, and in this way, by direct transmission, he fills the formative "mycetom" with "symbionts." The "mycetom" thus formed, he continues, ultimately fills the entire cavity of the blastocœle.

The circumstances which led to the suspicion that granular inclusions in the "mycetocytes" are of a biophytic nature form very interesting history. Blochmann (1886) appears to be the first to recognize the normal presence of intracellular "organisms" in eggs and body tissues of insects. The material he originally worked with consisted of ants and wasps. He noted that bacterialike bodies were present both in the eggs and in the follicular epithelium, and he suspected that they were probably bacteria, although, due to difficulty in working with that material, he very cautiously refrained from committing himself to a definite statement as to their exact nature. He was more confident of his results in his papers published during the following two years (Blochmann, 1887, 1888) for, as he noted, he had found more adequate material for this kind of investigation in *"Periplaneta orientalis"* and *"Blatta germanica."* As in the ants and wasps he worked with, he also found rod-shaped bodies in the fatty tissue and in the eggs of blattids. His conclusions were more straightforward in the latter case as to the suspicious bacterial nature of these cellular inclusions; he based his contention on their reaction to the various reagents and stains which he used, their multiplication by fission, and their method of infection through the egg. He also attempted to cultivate the "organisms" in beef-peptone-gelatine-agar media, but his results, he reported, were unsatisfactory.

Soon after this author, Wheeler (1889) noted similar bacterialike bodies in the periplasm of *Blattella germanica* Linnæus.

Cholodkowsky (1891) and Heymons (1895, pp. 78, 79) were also among the earlier workers who confirmed Blochmann's observations.

Following the lead of Blochmann, other investigators, influenced either directly or indirectly by his findings, soon took up the subject of "symbiosis" between insects and intracellular "organisms," and the aphids were among the first groups to be

worked with. Krassilschtschik (1889, 1890) appears to be the first to work on this family in that connection. His papers were based on investigations on twenty species of aphids. He noted the presence of bacilli which, he suspected, have some relation with the "symbiotic organs." He further noted that "organisms" were located only "entre la couche des cellules adipeuses en dessus et le pseudovitellus en dessous," to the exclusion of all the other organs. He did not recognize the cytoplasmic inclusion of the "mycetocytes" as microörganisms. He termed these bacilluslike bodies "biophytic bacteria," and pointed out that they were not parasites for the reasons that the tissue in which they were found was normal, that they were transmitted directly from one generation to the next, and that they were present in every representative of the species, even in the embryo. He further observed that the development of the mycetom in the embryo began at a very early stage, before the formation of the anlagen of the genitalia which fact, he pointed out, indicated the great importance of this organ in the vital processes of the aphid.

Henneguy (1904), with very little original evidence and drawing mainly from an unpublished work and figures of Balbiani, characterized the green, granular mass which infects the eggs of aphids at an early stage as "microörganisms" and, further, stated that the follicular epithelium, especially the thickened portion which adjoins the posterior pole of the egg, has something to do with the presence of these bodies, for he noted the incursion of the "symbionts" from that source through the gap left by the blastodermal wall.

Pierantoni (1909, 1910, 1911, 1911a) and Šulc (1910), working independently of each other, appear to have been the first to make an effort to prove experimentally that the cytoplasmic inclusions in the "mycetocytes" of aphids are living microorganisms, which are related to the yeasts. The evidence they presented appears to be principally morphological, these bodies resembling the Saccharomycetes in shape and in their method of division by either budding or fission. These authors also worked on other Homoptera. Šulc proposed the name "mycetom" for the organ; and "mycetocyte" for the individual "symbiotic" cell.

Buchner (1912, 1921) published two exhaustive treatises on "symbiosis," not only in aphids, but also in coccids, aleyrodids, psyllids, Cicada, cicadellids, blattids, Hymenoptera, Coleoptera, and Lepidoptera. He gave us a comprehensive review of our

present knowledge of "symbiosis" between insects and yeastlike or bacterialike "organisms," in addition to data based on his own work. His observations on the embryonic history of the "symbionts" have already been noted above. He described two species of "symbiotic organisms" in aphids, one of them being, according to him, new to science.

Up to the present time no worker has, to my knowledge, reported a single instance where the intracellular "symbionts" in aphids have been successfully cultivated in artificial media. At least three papers, based on attempts to propagate these "organisms," have been published: One by Pierantoni (1910), who claimed to have obtained colonies and to have observed that many of the organisms multiplied by budding on potato slices; and two later reports by Peklo (1912, 1916) who, likewise, recorded supposedly successful results in inoculation of artificial media, and isolation of pure cultures of the "symbionts" in dilute beef bouillon to which 6 per cent sucrose was added and in agar media made from this solution. Neither of these two authors appears, however, to have furnished adequate evidence to prove the identity of their artificially obtained microörganisms with the "microörganisms" of the aphid "mycetocytes." The difficulty in experiments of this nature appears to lie in at least three causes: (*a*) The microörganisms, if they be such, are probably so specialized in their physiological relations to their host that they cannot live outside the living aphid tissue; (*b*) proper laboratory technic in cultivating these "symbionts," both as to the kind of media used and the manipulation of the organisms, perhaps still remains to be discovered; and (*c*) the difficulty in proving by ordinary bacteriological methods that the organisms obtained in the artificial culture are identical with the cytoplasmic inclusions in the "mycetoms" of aphids, and not contamination from other sources. The last cause is a particularly troublesome obstacle, as I have experienced in my own work during the summer of 1920, in which I succeeded in obtaining yeastlike microörganisms in culture tubes that I had inoculated from various species of aphids, but failed to establish positive proof of their identity with the "symbionts." One method which has been suggested in connection with this work, and which I have not had occasion to use, is to "fish out" individual "microörganisms" with the aid of microdissection apparatus and make direct observations in hanging drops.

As a whole, the assumption that the granular cytoplasmic inclusions of these specialized cells in aphids are symbiotic

organisms has been based mainly on indirect evidence. To the morphological proof offered principally by Šulc and by Pierantoni, the following facts may be added: (*a*) As I shall discuss presently, the "symbionts" which are apparently in a quiescent state in the follicular epithelium are at once given a sudden start in their multiplication as soon as the barrier of the dense "Keimhautblastem" disappears at the posterior pole of the egg, thereby bringing the yolk material in contact with them. The granular bodies which are lodged in the follicular epithelium are apparently stimulated by the egg yolk, their rapid multiplication at this time being manifest in the marked swelling of the follicular epithelium near the posterior pole of the egg and the rapid incursion of the "microörganisms" from the former into the latter. This sudden response strongly suggests that these granular inclusions are microörganisms, which begin to grow and divide rapidly as soon as a rich food supply is made available to them. (*b*) Another important peculiarity which leads one to suspect strongly that these inclusions are microorganisms is the marked attraction toward the mass in which they are contained by certain cleavage nuclei, in the earlier stages of the development of the egg. This behavior of certain nuclei or cells of the body proper, as in the case of phagocytosis previously described by Metschnikow (1901),[4] is displayed in the presence of foreign protein.

The basis for the supposition that these "microörganisms" are of a symbiotic nature, as worked out by earlier investigators, has been enumerated by Baumberger (1919, p. 72) and later recast, in a more satisfactory form, by Glaser (1920). I quote from the latter:

> (*a*) Every individual of a species is infected.
>
> (*b*) The infection produces changes in the host cells, but these are harmless.
>
> (*c*) The infection routes and methods of localization, while different in different hosts and symbionts, follow very definite courses within a species.
>
> (*d*) The microörganisms are numerically controlled by the host, never increasing up to a point where they may prove fatal.
>
> (*e*) The microörganisms within the insects obtain nourishment and protection from drastic temperature and drought conditions.

In the case of the coccids, Brues and Glaser (1921) have recently reported that they have succeeded in cultivating the

[4] This comparison of the behavior of the cleavage nuclei with phagocytosis was suggested to me by Professor Wheeler.

"symbionts" of *Pulvinaria innumerabilis* Rath. In this species there are no specialized "mycetoms" or "mycetocytes," as in the aphids, the supposed microörganisms in the former being found in the adipose tissue. These authors have undertaken to prove the identity of the microörganisms they obtained in their culture media with the coccid "symbionts" by the following criteria: The presence of apparently the same microörganisms in practically all the culture tubes inoculated; the morphological similarity between these microörganisms and the "symbionts" in the body of *Pulvinaria,* and the fact that the former were "recovered in such a large proportion of the cultures," which "left little doubt as to the identity of the two;" and the positive precipitin reaction in the rabbit serum. They have also made a study of the microörganisms they obtained, and noted that they secrete proteolytic, lipolytic, and diastatic enzymes, which, they suggest, may have some relation to their symbiotic nature. It is to be hoped that the work so well begun by these two authors will serve as a stimulus to other investigators in the way of finding a more adequate method of satisfactorily solving the more exact nature of these puzzling bodies in aphids, as well as in other insects.

MATERIAL AND TECHNIC

The material used in the present work was drawn mainly from one species, *Macrosiphum tanaceti* Linnæus. My choice of this aphid was prompted by the fact that during the growing season it is available in great abundance on its host plant, *Tanacetum vulgare* Linnæus (Compositæ), which is a very common weed in the immediate vicinity of the Bussey Institution, thus insuring an adequate supply of specimens. Furthermore, *Macrosiphum* is one of the Aphidinæ,[5] a group which systematists consider, "with the exception of the Mindarinæ, * * * as by far the most primitive" subfamily of the Aphididæ (Baker, 1920). It was believed that by working with a member of this subfamily there would be a better chance to observe characters which might otherwise be modified, obscured, or lost as a result of specialization and that more satisfactory generalized conclusions could thus be reached.

The following species, a large number of which are members of the subfamily Aphidinæ, have also been studied, on a less

[5] Baker (1920) has placed the genus *Macrosiphum* under the subfamily Aphidinæ, tribe Aphidini, subtribe Macrosiphina.

extensive scale, however, in the hope of securing supplementary as well as confirmatory data:

APHIDINÆ [6]

Anoecia corni Fabr.*
Anoecia querci Fitch.*
Lachnus dentatus Le Baron.
Longistigma caryæ Harris.
Drepanaphis acerifolii Thomas.
Drepanosiphum platanoides Schrank.*
Aphis rumicis Linn.*
Macrosiphum œnotheræ Oestlund.
Macrosiphum pisi Kalt.
Macrosiphum rosæ Linn.
Macrosiphum rudbeckiæ Fitch.
Macrosiphum sp. on *Cichorium intibus* Linn. (Compositæ).
Macrosiphum sp. on *Sicyos angulatus* Linn. (Cucurbitaceæ).
Myzus persicæ Sulzer.

ERIOSOMATINÆ

Eriosoma lanigera Hausm.

In addition, unidentified aphids from the following host plants were studied:

Berberis vulgaris Linn. (Berberidaceæ).
Celastrus scandens Linn. (Celastraceæ).
Lythrum salicaria Linn. (Lythraceæ).
Shepherdia (*Elæagnus*) *argentea* Nutt. (Elæagnaceæ).
Solidago virgaurea Linn. (Compositæ).
Viburnum sp. (Caprifoliaceæ).

Biological observations have been made both in the field and in the insectary. For life-history work, each aphid was kept separate on individual food plants by inclosing it with a lamp chimney the top of which was covered with fine muslin. In order to avoid duplications in recording the rate of parturition, the newly born nymphs were destroyed after a count of them had been made.

For a study of aphid embryology, two series are employed; namely, (*a*) fresh material and (*b*) fixed and stained preparations. Examination of fresh material has been found to be essential in the study of the sequence of eggs in the ovarioles and for observations of the normal structure of the ovaries and their contents. Ringer's solution has been employed as dissecting medium. On account of the scarcity of yolk in the parthenogenetic eggs, the constituent parts can be recognized fairly distinctly in the fresh preparations under the microscope until the later part of the blastoderm stage. In *Macrosiphum tanaceti* the green color of the mass of "symbiotic organisms" makes the formative mycetom stand out prominently in contrast

[6] The arrangement in the present list follows the generic sequence in Baker's (1920) paper. Names marked with an asterisk indicate species for the identification of which I am indebted to Miss Edith M. Patch.

with the rest of the egg, which is pale yellowish transparent, thus enabling the observer to trace the general behavior of the developing organ. Very satisfactory results have been obtained by treating fresh material with Schneider's acetocarmine, especially in the study of cellular contents of the germaria, young eggs, and eggs in earlier cleavage stages. This stain brings about an excellent differentiation of the nucleus and of the cytoplasm, but the tendency of the acetic acid contained in the solution to render somewhat opaque an originally semitransparent tissue makes it unsuitable for use in connection with older aphid embryos, where one has to deal with bulkier objects. Nor can it be used to advantage in connection with the study of the "symbiotic organs," as the stain destroys the original green pigment in the mass of "microörganisms." The entire egg constituents are thus reduced to the same color, and the "symbiotic organs" which are clearly discernible in the fresh material become obscured.

For making permanent preparations, I have used two groups of fixatives in which either picric acid or bichloride of mercury forms the principal active part. Of the former group, I have tried Kleinenberg's picro-sulphuric solution and Bouin's picro-formol with acetic acid; and of the second, Gilson's mercuro-nitric mixture, Carnoy and Lebrun's fluid, and Webster and Phillips's (1912) sublimate-acetic-alcohol mixture. I have found that for general purposes the mixtures containing corrosive sublimate are more satisfactory, in that they have a stronger penetrating power and thus produce better preservation and less distortion in the tissues. Furthermore, material treated with these fixatives takes the stain much more sharply, especially when aniline dyes are used. Carnoy and Lebrun's fluid is too violent in its reactions and, for this reason, it has been employed in my work to a limited extent only, in connection with winter eggs, the thick chorion of which is not readily permeable to the milder solutions. Gilson's fluid, heated to about 80° C., has been used much more extensively than any of the other mixtures.

Viviparous aphids of different instars are brought into the laboratory and the abdominal walls are punctured once or twice with very fine needles, care being taken in performing the operation not to make the wounds too large as otherwise the body contents would ooze out. The specimens so treated are then dropped into the fixing fluid which has been previously heated to about 80° C. The aphids are almost instantly killed on contact with

the liquid at this temperature and penetration by the fixative is also greatly enhanced.

Except where the embryos are to be mounted in toto, no advantage is gained by dissecting them out of the mother and embedding them separately. In fact, the advantages appear to be in favor of sectioning the entire aphid, for then considerable time is saved in at least three ways; namely, in being spared the trouble of dissecting; in that each embryo does not have to be embedded separately; and in the fact that in sectioning, from about thirty to a hundred eggs and embryos, depending upon the instar of the mother (see Table 1), are cut at one time from each block, where otherwise there would have been only one. While it is true that in sectioning the entire aphid, it is impossible to control the orientation of the abdominal contents, still I have found that in practically all my sections some of the eggs and embryos are cut on an exactly sagittal, frontal, or crosswise plane, while the rest present an oblique or tangential aspect. The latter are very useful in comparative studies of relationships and structure of the internal organs. A further advantage in preparing sections of whole aphids is that the normal bearing of the eggs, embryos, and the ovaries in their relation to the adjoining parts of the body is kept intact, as in its natural condition. Among the more serious disadvantages in connection with preparing individual embryos separately for sectioning is the fact that there is a noticeable amount of distortion of the different organs, due probably to the extreme delicacy of the embryonic tissue which makes it easily affected by too much handling and by direct exposure to the various reagents used.

Paraffines of two degrees of hardness have been used for embedding, one with melting point at 46° to 48° C., and the other, at 54° to 56° C. The use of clove oil as a clearing agent prior to the transfer of material to the paraffine bath was abandoned after several trials on account of its tendency to render the tissues too brittle. Xylene was found to be much more satisfactory. About one hour in the soft paraffine and three to four hours in the hard has been found sufficient for thorough impregnation of the objects. In preparing the blocks for sectioning, I have found that I can work much faster and obtain much more satisfactory results by using small watch glasses as molds, instead of paper boxes or other devices.

The objects are cut from 3 to 10 micra thick. Most of them are prepared at about 7 micra, this thickness having been found

to be the most suitable for ordinary purposes. Frontal, sagittal, and a few cross sections have been made. Mayer's albumen has been used in fixing the paraffine ribbons onto the slides.

Borax carmine and alum cochineal both gave satisfactory results with embryos prepared in toto. For staining sectioned material, Ehrlich's acid hæmatoxylin, without counter stain, was largely employed and found to be highly suitable for general histological work. This solution gives a very good differentiation, with soft color contrast and, unlike Heidenhain's iron hæmatoxylin, does not obscure any of the tissues by staining them too black. Ehrlich's hæmatoxylin has the further advantage of acting relatively more quickly, and it yields a much larger percentage of good or otherwise usable slides than all the other stains I have used. Very beautiful preparations have also resulted from the use of safranin and gentian violet, according to the method described by Guyer.[7] For a more detailed study of cell structures, sections 3 to 5 micra thick are stained in Heidenhain's iron hæmatoxylin.

PARTHENOGENETIC EMBRYOLOGY AND HISTORY OF THE "MYCETOM"

GENERAL CONSIDERATIONS OF THE OVARIES

In as much as all the embryonic stages in parthenogenetic aphids are passed in utero, it seems advisable, for the sake of a more adequate understanding of some of the topics in the discussion which follows, to include in the present paper a description of the ovaries. The description herein presented is based on *Macrosiphum tanaceti* and on an allied species, *M. rosæ*.

The opening of the vagina is bounded externally by two large plates and a pair of smaller sclerites which, to use the terminology adopted by Tullgren (1909), are as follows: (*a*) Dorsally, a round-top, elongately subconical anal plate (Plate 1, fig. 1, *ap*), which is about one and one-fourth times as long as its dorsoventral basal diameter and the entire surface of which is sparsely set with elongate rows of abrupt spines and, here and there, a few long hairs, resembling in this respect the superficial characters of the style, or abdominal cauda; (*b*) ventrally, an acutely subconical genital plate (Plate 1, fig. 1, *gp*), which presents a coarsely punctate surface, sparsely set with moderately long hairs; and (*c*) laterally, on each side of the vaginal slit,

[7] Guyer, M. F., Animal Micrology, revised ed. Chicago, The University of Chicago Press (1917) 234.

a rudimentary gonapophysis (Plate 1, fig. 1, *gon*), which is considerably smaller than the anal plate and which possesses the same superficial characteristics as the genital plate.

From that opening, or slit, the vagina extends anteriorly to about the junction of the genital plate and the adjoining anterior abdominal sternite. The vagina, when empty, is compressed dorsoventrally, so that in cross section (Plate 2, fig. 18) the dorsal and the ventral vaginal walls are observed almost to touch each other. The greatest width of the vagina in this condition is about 126 micra. The wall consists of three layers: (*a*) A thin, innermost chitinous intima, which is continuous with the exoskeleton of the insect (Plate 2, figs. 16, 18, *int*). (*b*) A thick hypodermis (Plate 2, figs. 16, 18, *hyp*), which constitutes the main portion of the wall. This layer is about three or four times as thick around the rim of the vaginal slit as in the adjoining anterior portion. The hypodermal cells are elongately or abruptly columnar and, on account of the marked differences in the length of these cellular constituents, the ental surface of the hypodermis presents a rugged appearance. The nuclei are large in proportion to the size of the cell, subglobular or abruptly subellipsoidal, with a distinct nucleolus and numerous scattered chromatin granules. The cytoplasm is highly vacuolated. (*c*) A basement membrane, which serves as support for the muscles which operate the vagina. The muscular equipment of this organ appears to be most strongly concentrated around the vaginal slit. The muscles in this region are characteristically spindle-shaped, and measure about 40 to 45 micra long, being about twice the length of the muscles found in association with the more anterior portion of the vagina. They have their origin at the ental walls of the anal plate and of the rudimentary gonapophyses. The function of these muscles is apparently to open and close the vaginal slit. One important point to consider in connection with the vagina is the absence of the spermatheca and of the colleterial glands which, in amphigonous individuals, arise as an evagination of the vaginal wall. This peculiarity of the ovaries in parthenogenetic aphids was first reported by von Siebold (1839); I have already called attention to this fact in an earlier paper (Uichanco, 1921).

Two rather short oviducts are present. They measure approximately 70 micra in their greatest cross-sectional diameter. There is nothing of any particular interest in this region, except that the chitinous intima which lines the ental surface of the vaginal wall is absent here. The epithelial wall of the oviduct is

considerably thinner than the hypodermis of the vagina. The former is composed of flat, subpolygonal cells, with large globular nuclei and highly vacuolated cytoplasm. The muscular equipment of the oviduct is very much sparser than that of the vaginal wall.

There are twelve ovarioles, or ovarian tubules, arranged in two equinumeral groups, each group joining posteriorly one of the two oviducts. A similar number has been observed by Balbiani (1869) and by Stevens (1905) in connection with other species of aphids. Each ovariole terminates anteriorly in a filament (Plate 3, figs. 21, 22, *fil*), which is connected with the dorsal diaphragm of the body of the mother at about the junction of the metathorax and the first abdominal segment. The terminal filament has an average diameter of 1.5 micra. It is noncellular and is apparently continuous with the investing membrane which envelops the ovariole. Entally a mass is visible containing numerous minute granular inclusions, the nature of which I was unable to determine satisfactorily on account of their extremely small size. These granules stain rather deeply in Ehrlich's acid hæmatoxylin, and in this respect are very suggestive of nuclear material. The function of the terminal filament is apparently to serve merely as mechanical support for the ovariole. This interpretation was first proposed by Stein (1847) in connection with similar structures in other insects. In aphids, this structure was apparently first noted by Huxley (1858).

The ovariole is a tubular structure which, owing to the presence of eggs in a longitudinal row from the subapical portion to the posterior end, presents a submoniliform aspect. The wall is composed of an ental epithelium, with the exception noted below, and an ectal investing membrane. The ovariole has two main divisions: the germarium, or terminal chamber (Plate 3, figs. 21, 22, 23, *gm*), and the vitellarium (Plate 3, figs. 21, 22, 23, *vit*). The former was first described by Leydig (1850) in viviparous aphids. It is subpyriform or subglobular, with the epithelium of the anterior and lateral walls reduced to a very thin membrane in which the cells show evidence of being widely stretched. The wall at the posterior end, on the other hand, is about five to ten times as thick as are the anterior and lateral walls. There seems to be reasonable ground to suppose that the thinner epithelium constitutes the lining of the terminal chamber, which is passive in its nature and takes no active part in the formation of the follicular epithelium of the

egg; the thicker epithelial lining represents the mass of cells from which arises the latter tissue.

The vitellarium constitutes that portion of the ovariole into which the ovum is pushed upon extrusion from the germarium. The only static part of the wall of the vitellarium is the ectal investing membrane, in as much as the epithelial lining is represented by the follicular epithelium of the egg and the latter accompanies the egg or embryo in its downward migration into the oviduct. This state of affairs is partially in harmony with that obtaining in other insects in general, as characterized by Comstock (1920, pp. 158, 159) in the following words:

> * * * the epithelium lining of the ovarian tube becomes invaginated between the eggs in such a way that each egg is enclosed in an epithelial sac or egg-follicle, which passes down the tube with the egg. There is thus a tendency to strip the tube of its epithelium, but a new one is formed.

This view is confirmed by the aphid material I have examined, except for the fact that from Comstock's statement one would judge that the epithelial lining is stripped off along the entire length of the vitellarium. While this may be the case in certain other insects, it certainly is not in aphids, wherein the apportionment of epithelial lining to the egg takes place only at the anterior end of the tubule. The egg thus provided with a follicular epithelium passes posteriorly through the lumen of the vitellarium, the wall of which, to a greater extent, at this caudal portion consists only of an investing membrane and is devoid of an actual epithelial lining. The latter is represented by the egg follicle which from time to time, as one egg passes down after another, comes momentarily in contact with the investing membrane. Thus, strictly speaking, the epithelial lining of the vitellarium is stripped only from the anterior portion of the tubule, where it is continually replaced by proliferation of the epithelial cells at this region.

The embryonic life of the parthenogenetic aphid is spent in the vitellarium. Ovulation begins to occur early, even before the birth of the mother. The number of eggs and embryos in each vitellarium increases with the age of the mother, the number reaching its maximum limit some time after the latter has reached the adult stage. Since, when a young egg is extruded, the older ones are pushed posteriorly, the eggs and embryos in the vitellarium become arranged anteroposteriorly in a linear series in an ascendant chronological gradation.

SUMMARY

The ovary of a parthenogenetic *Macrosiphum* consists of a vagina, which is connected anteriorly with two short oviducts; and there are twelve ovarioles, or ovarian tubules, arranged in two equinumeral groups on each of the latter. Each ovariole is supported distally by a slender noncellular terminal filament, which is fastened anteriorly to the dorsal diaphragm of the insect, at about the junction of the metathorax and the first abdominal segment. The ovariole consists of a germarium, or terminal chamber, in which the oöcyte is lodged prior to extrusion; and a vitellarium, in which the embryonic life of the parthenogenetic aphid is passed. The eggs and embryos present in a vitellarium increase in number progressively with the age of the mother, and are arranged anteroposteriorly in a linear series in an ascendant chronological gradation.

DEVELOPMENTAL STAGES OF THE PARTHENOGENETIC EGGS AND EMBRYOS, IN THEIR RELATION TO THE AGE OF THE MOTHER

In an earlier publication (Uichanco, 1921) I cited the observations of Ewing (1916) on *Aphis avenæ* Fabricius and of Webster and Phillips (1912) on *Toxoptera graminum* Rondani, both of which papers reported cases of parturition occurring in what were thought to be nymphal forms of parthenogenetic females, as examples of pædogenesis in aphids. As has been satisfactorily explained by Turner and Baker (1915) and by Baker and Turner (1916a), both of which references I had overlooked in the preparation of my previous paper,[8] such aphid "nymphs" as have been observed to give birth to young are in reality adult "intermediates;" that is, individuals which have undergone the full number of molts and which possess all the characters of the alate adults, except that the wings are rudimentary. The rudimentary wings, as these authors have pointed out, have been mistaken for wing pads, and hence the erroneous interpretation. This citation in my earlier paper in connection with pædogenesis is, therefore, corrected here.

It has impressed me, however, that the methods heretofore employed in determining the nature of reproduction in aphids have been very superficial and unsatisfactory. The birth of young from viviparous females, which investigators have so far

[8] I am indebted to Dr. A. C. Baker, of the Bureau of Entomology, United States Department of Agriculture, for kindly calling my attention to this oversight.

taken into consideration only in this connection, does not correspond to the phenomenon of egg deposition as this process is known in amphigonous individuals, but represents the conclusion of the embryonic life of the parthenogenetic aphid. The intrauterine development of the embryo, which corresponds to the incubation period of zygogenetic eggs, has not been taken into account. In neglecting this part of the aphid's ontogeny, we are using an imperfect criterion, and whatever conclusions we may draw therefrom are, of course, erroneous. With these premises in view, I have made an attempt in the present work to determine both qualitatively and quantitatively by dissection of individuals of definitely known ages the probable extent to which pædogenesis occurs in aphids. Observations had to be limited to one species, *Macrosiphum tanaceti,* living bred material having been used in all cases. Ringer's solution proved very satisfactory for examination media. Dissection was made under a pair of binoculars by means of tips of No. 0 steel insect pins which had been mounted on handles and ground to a fine point. An effort was made to keep the succession of eggs in the individual vitellaria intact, but on account of the extremely delicate nature of the ovarian walls, their accidental laceration was often unavoidable. The slightest rupture of the ovariole enables one or more of the eggs to escape, and since they thus disturb the sequence of the contents, that particular material is rendered useless for the purpose of experiments like the present ones. Owing to this difficulty in dissection, only one or two, and often none, of a total of twelve ovarioles present in each individual, can be studied.

Observations were made in connection with mothers of the following instars. The letters correspond to those at the head of each column in Table 1.

A. Nymphs of the first instar, immediately after birth. The eggs present in the vitellaria at this age represent the total ovulation during the prenatal stages of the mother.

B. Nymphs of the second instar, immediately after the first molt. The eggs at this age represent the total ovulation during a period which begins with the prenatal stages of the mother and ends with the completion of the first instar.

C. Nymphs of the third instar, immediately after the second molt. The eggs at this age represent the total ovulation from the prenatal stages of the mother to the completion of the second instar.

D. Nymphs of the fourth instar, immediately after the third molt. The eggs at this age represent the total ovulation from the prenatal stages of the mother to the completion of the third instar.

E. Adults, immediately after the fourth molt. The eggs at this age represent the total ovulation from the prenatal stages of the mother to the completion of the fourth instar.

F. Adults, after they had given birth to the first young.

Total ovulation from the prenatal stages of the mother through the adult stage could be determined only approximately after the birth of the first young in the present experiments, for two reasons: (*a*) The individuals used were mostly bred en masse, which fact made it impracticable to determine which mother had given birth to the young; and (*b*) even in the case of individuals bred singly, the tendency of the walls of the ovarian tubules to rupture during manipulation made it almost impossible to determine, by comparison, from which one of the twelve vitellaria the young nymph had been extruded. In cases of mothers confined individually it will probably be possible to follow the rate of ovulation during the adult stage after the birth of the twelfth young and the multiples of this number. This number is taken since, as I have already noted, there are twelve ovarian tubules in the parthenogenetic *Macrosiphum tanaceti*. The corresponding eggs in each tubule in one individual are approximately of the same age and are presumably born in close succession to one another. Thus, for every twelve young born, there is to be made an allowance of one when the total contents of each ovarian tubule are counted.

Another method of determining ovulation in the adult stage is to count the total number of young born from the mother during the entire period of fecundity of the latter. The percentage of mortality of the embryos in utero, so far as I am able to determine by dissection, is nil or almost negligible. In as much as the latter two experiments were not started until rather late in the season, sufficient data have not been obtained for adequate discussion in the present paper.

The findings from dissections of individuals of known ages are presented in Table 1. This table shows clearly the number and the comparative degree of develoment of the eggs in each ovariole at the conclusion of the corresponding instar of the mother as well as the developmental history of each individual egg as observed at the conclusion of each maternal instar.

TABLE 1.—*Comparative stages and individual developmental history of eggs in one vitellarium at the conclusion of each instar in parthenogenetic Macrosiphum tanaceti.*

Egg No. (in postero-anterior sequence within vitellarium).	A. At birth.	B. First molt, about 48 hours after birth.	C. Second molt, about 30 hours after first molt.	D. Third molt, about 48 hours after second molt.	E. Fourth molt, adult, about 48 hours after third molt.	F. Adult, 3d day, about 80 hours after fourth molt.
I	Blastomeres differentiating, or blastoderm fully formed. "Germ cylinder" not evident, or beginning to proliferate.	Ventral plate extending to entire length of egg. No formation of metameric segments.	Beginning of metameric segmentation or formation of anlagen of appendages.	Embryo with differentiated appendages and nervous system. Immediately prior to, or in process of, or immediately after rotation of embryo.	Embryo with fully developed appendages, body wall and internal organs.	Full-grown young, being born.
II	Cleavage nuclei at periphery, preparatory to formation of definitive blastomeres.	Blastoderm. Ventral plate about one-third total length of egg.	Ventral plate extending to entire length of egg, hooked at anterior end. No metameric segmentation.	Anlagen of appendages. Prior to rotation of embryo.	Embryo soon after rotation. Some with body wall, appendages and internal organs well formed. About two-thirds length of I.	Well-developed young, about two-thirds length of I.
III	Egg freshly extruded, or with fully formed cytoplasmic mass.	Blastoderm newly formed. No "germ-cylinder."	Ventral plate about one-fourth total length of egg. "Germ-cylinder" beginning to proliferate.	Beginning of metameric segmentation.	Anlagen of appendages.	Embryo after rotation. Appendages differentiated; also body walls and internal organs. Hind legs extending only to abdominal cauda.

IV		Egg prior to cleavage, or first cleavage, or cleavage nuclei migrating to periphery.	Differentiation of blastomeres, or migration of cleavage nuclei to periphery.	Blastoderm. Ventral plate one-third to one-half total length of egg.	Ventral plate two-thirds to total length of egg. Some with beginning of metameric segmentation.	Embryo prior to rotation; anlagen of appendages.
V			Egg freshly extruded, or egg more or less well developed with large cytoplasmic mass.	Differentiation of blastomeres.	Blastoderm. Ventral plate beginning to proliferate, or one-half total length of egg.	Beginning of metameric segmentation.
VI				Egg freshly extruded.	Differentiation of blastomeres, or blastoderm and proliferation of "germ-cylinder."	Ventral plate about as long as egg.
VII					Egg being extruded, or first cleavage.	Ventral plate one-third to one-half total length of egg.
VIII						Differentiation of blastomeres. Egg very elongate and narrow.
IX						Egg freshly extruded, or fully formed, with large mass of cytoplasm.

DISCUSSION AND CORRELATION

The following deductions are made from an analytical study of Table 1:

1. Ovulation in parthenogenetic forms of *Macrosiphum tanaceti,* and apparently of many other species of aphids also, begins at a period when the mother is still in the embryonic state. This fact has been known for a long time. Metschnikow (1866) appears to be the first to report similar observations, in the following statement:

> Noch während des embryonalen Lebens der Aphisembryonen fängt die Entwicklung der neuen Generation an, wobei sie so weit geht, dass bei den, zum Gebären reifen Embryonen sich bereits zwei Keimfächer in jeder Eierstockröhre befinden, so dass das unterste ein Pseudovum mit ganz entwickeltem Blastoderm in sich einschliesst.

In general, my observations agree with his, except in the figure he gave for the number of eggs in the ovariole of the nymph at birth, as I am pointing out in the following paragraphs. He worked on *"Aphis pelargonii"* and *"Aphis rosæ,"* and it is quite probable that the discrepancy between his figure and mine is due to the difference in our experimental material. It is also probable that he had overlooked the very young oöcyte, which is just being extruded from the germarium at this age of the mother.

2. In all the newly born nymphs dissected, three eggs were found on the average in each vitellarium. From the very small size and relative scarcity of the surrounding cytoplasm, it is apparent that egg III [9] has been extruded from the germarium at about the conclusion of the prenatal stages of the mother or while she was being born. For this reason, only eggs I and II are really extruded during the embryonic stages of the mother. Thus in the twelve ovarioles present in each individual mother, a total of twenty-four eggs are extruded from the germaria before she is born.

3. The number of eggs extruded from each germarium during the various maternal instars and the time required in each case are given in Table 2.

4. As shown in Table 2, a much larger percentage of eggs is extruded during the preadult than during the adult stage of the mother. The total number of young born from each parthenogenetic female during her period of fecundity has never been

[9] Roman numerals refer to egg numbers, as indicated in the first column in Table 1.

counted directly in the case of *Macrosiphum tanaceti.* Webster and Phillips (1912), however, who did very careful experimental work, based on a large number of individuals, on a closely allied species, *Toxoptera graminum* Rondani, published the following statements on the fecundity of viviparous forms:

> The maximum number of young produced by those born from March to the middle of June is 69, the average number for each individual for this period being 30.3; the maximum for those born from the middle of June until the middle of August is 93 young, the average number for each individual being 25.3; the maximum for those born after the middle of August is 66 young, the average for each individual being 24.

TABLE 2.—*Comparative rate of ovulation in different maternal instars, computed from Table 1.*

Stage of mother.	Eggs extruded from germarium.	Approximate time requirement.
		Hrs.
Prenatal	2	[a] 55
First instar	2	48
Second instar	1	30
Third instar	1	48
Fourth instar	1	48
Adult	[b] 2	80

[a] Calculated average from Table 3; 2 days 7 hours = 55 hours.

[b] Adults dissected after birth of first young. This number does not take into account possible subsequent ovulation.

The maximum obtained by these authors for all their experiments is 93 young. So far as I have carried the experiments on *Macrosiphum tanaceti,* which has reached the adult stage and begun to give birth to her young, the number of eggs present in the vitellarium at any one time is 9, which makes a total of 108 in the twelve ovarian tubules. If all of these are born as young, this maximum figure obtained from dissection of *Macrosiphum* is rather close to the maximum number of young observed by Webster and Phillips on *Toxoptera graminum.* After expulsion of the first young from the vitellarium, therefore, it is doubtful if ovulation proceeds to any appreciable extent beyond the maximum figure I have obtained. Taking 9, then, as an approximately close estimate for the maximum number of eggs which pass into each vitellarium of a parthenogenetic *Macrosiphum tanaceti,* we see from Tables 1 and 2 that ovulation occurs mainly in the embryonic and nymphal stages of the mother, only about 25 per cent of the total being extruded during the adult stage.

5. Cleavage and subsequent development proceed soon after the eggs are extruded from the germarium.

6. By following egg No. I in Table 1 to the conclusion of each maternal instar, it will be noted that:

a. The latest stage reached by the oldest egg in the vitellarium at birth of the mother is the blastoderm stage in which the germ cylinder is not yet in evidence or is just beginning to proliferate (Plate 1, fig. 38 or 39; or Plate 6, fig. 40).

b. The formation of metameric segments and the appearance of the anlagen of the appendages (Plate 7, fig. 44; or Plate 8, fig. 49) do not occur until about the conclusion of the second instar.

c. At about the conclusion of the third instar, the oldest embryo has the nervous system, mouth parts, legs, and other parts of the body well differentiated (Plate 9, fig. 51; or Plate 10, fig. 53; or Plate 11, fig. 56; Plate 12, fig. 57). The invaginating stomodæum and proctodæum are fully in evidence. Rotation, or orientation, of the embryo does not occur until about this time. By inference, it also follows that rotation of the embryo does not occur until after the parts indicated above are differentiated.

d. At the conclusion of the fourth instar, that is, as the mother is passing into the adult stage, the oldest embryo in her ovariole has fully developed appendages, body wall, and internal organs (Plate 1, figs. 6, 7, 8), but is noticeably smaller than the corresponding ones which are about ready to be born in older mothers (Plate 1, fig. 10).

7. In as much as the extrusion and subsequent development of about 75 per cent of the parthenogenetic eggs occur during the preadult stages of *Macrosiphum tanaceti,* only about 25 per cent of the total ovulation taking place in the adults, pædogenesis may be considered as a normal method of reproduction in this and probably in other species of aphids. This point was not brought out in my previous paper (Uichanco, 1921). The occasional observations on parturition during the apparently nymphal stage of aphids is really not an adequate basis for judging the extent of pædogenetic development which is going on within the ovaries of the viviparous mother. The extrusion of the eggs into the vitellaria and the subsequent intrauterine development of the former while the mother is still in the embryonic or nymphal stage may be considered as satisfying the conditions for pædogenetic reproduction, irrespective of whether the young is born during the preadult stages of the

mother or later. The classical case of *Miastor metraloas,* discovered by Wagner (1862), and to which von Baer (1863) applied the name "pædogenesis," occurs in the form of a larva which gives birth to another larva. The general principle involved in the two types, represented by parthenogenetic aphids on one hand and by *Miastor* on the other, is the same, the difference in the visible manifestation of the end products being, so far as biological significance of pædogenesis is concerned, of minor importance. Or, as Wilder (1909, pp. 21 and 22), speaking in general terms of reproduction in animals, so aptly expressed it—

> the developmental history of an animal includes all stages from that of a fertilized egg (ovum) to that of the sexually matured adult, and is not in any way interrupted by the act of birth or hatching. These latter are purely external phenomena, and mark no important stage in the development of an animal, save in the line of certain necessary adaptations.

In order to avoid confusion of terminology, however, there appears to be a necessity for a new definition of the original term, and the following reclassification is hereby proposed:

I. PÆDOGENESIS (*pædo-*, or *pedo-*, from παῖς, παιδός, child, + genesis, from γένεσις, from root of γίγνεσθαι, to be born). Definition: That method of sexual reproduction whereby during the immature stages of the mother the ovum reaches a condition which enables it to begin a new germ-cell cycle.[10]

1. BISEXUAL PÆDOGENESIS.[11] Definition: That form of pædogenesis wherein the union of biparental elements accompanies reproduction. The preadult individuals in such cases "attain sexual maturity and produce eggs and sperms." Examples: *Bolina hydatina* (Ctenophora) and *Ambystoma tigrinum* (Chordata) (Shull, 1920, pp. 181–183).

2. UNISEXUAL (PARTHENOGENETIC) PÆDOGENESIS.[12] Definition: That form of pædogenesis wherein the development of the ovum takes place without fertilization. As I have pointed out in the foregoing discussion, in the viviparous forms, the extrusion of the ovum from the germarium and the subsequent intrauterine development thereof correspond in their biological significance to oviposition and the subsequent incubation of the eggs in the ovipara.

[10] The term "germ-cell cycle" is adopted here in the sense discussed by Hegner (1914, pp. 28–29).

[11] The idea of divisions 1 and 2 in the present classification is drawn from Shull (1920, pp. 181–183).

[12] The definition in my previous paper (Uichanco, 1921) to the effect that "pædogenesis is parthenogenesis occurring in the preadult stages of animals" is no longer tenable, because too involving, and should be corrected to conform with the present classification.

a. UNISEXUAL ECTOPÆDOGENESIS (*ecto-*, from ἐκτός, outside, + pædogenesis).[13] Definition: That form of unisexual pædogenesis wherein the externally visible manifestation of the end products, in the form of extraovarian extrusion of eggs or young, occurs during the preadult stages of the mother. Examples: Certain groups of Itonididæ, like *Cecidomyia* (in some of the species only) and *Miastor*.

b. UNISEXUAL ENTOPÆDOGENESIS (*ento-*, from ἐντός, within, + pædogenesis). Definition: That form of unisexual pædogenesis wherein the development of the ovum takes place in utero during the immature stages of the mother, and consequently the externally visible manifestation of the end products, in the form of extraovarian extrusion of eggs or young, does not occur until subsequently. Examples: Parthenogenetic Aphididæ.

At this juncture, Pérez's (1899) definition of metamorphosis (in the sense of successive abrupt changes undergone by an organism during the course of its development) as "une crise de maturité génitale" may be aptly considered. Giard (1900) has published a refutation of Pérez's views and raised the following questions:

Toute métamorphose est-elle accompagnée d'une crise de maturité génitale?

Toute crise de maturité génitale est-elle forcement accompagnée d'une métamorphose?

He makes an attempt to show that the foregoing two questions demand in general a negative answer, basing his arguments on the behavior of certain groups of animals, principally insects. His contention is that metamorphosis is not dependent on the development or nondevelopment of the genital organs, for in cases of parasitic or alimentary castration, the development of the rest of the body appears to proceed normally. He also makes an effort to show that the reverse is likewise true; that is, the development of the genital organs is independent of metamorphosis, using as his examples the fact that in neoteinic individuals of certain animals sexual maturity is reached before metamorphosis is completed and, further, in cases where alimentary castration is suppressed, as in the queen of Vespidæ,

[13] Von Baehr's (1863) "pædogenesis" and Hatschek's (1877) "pædoparthenogenesis," the latter term being used also by Henneguy (1904, pp. 259, 260), come under this heading. "Pædogenesis," as I have already noted, includes several other categories which have come to light since the original term was coined and, therefore, needs a revision in definition. "Pædoparthenogenesis" is unsuitable because ambiguous. The term would include both (*a*) and (*b*) in the present subdivision.

acceleration of development of genital organs takes place without a corresponding effect on metamorphosis. He also cites the case of parthenogenetically reproducing insects, which, he claims, have dispensed with their adult stage. He is, however, wrong in the case of the aphids. The viviparous generation of this family has not, as he says, suppressed the imaginal stage, for parthenogenetic individuals with fully developed wings are produced.

Neither Giard nor Pérez has defined the meaning of "*une crise de maturité génitale.*" From their discussion, however, it is apparent that what is meant is the condition whereby the reproductive organs begin to become functional; that is, when mature ova or spermatozoa begin to be produced. In this sense, sexual maturity in parthenogenetic aphids takes place soon after differentiation of the definitive ovarian tubules, when the insect is still in the prenatal stages. My own observations on parthenogenetic aphids appear to confirm the views of Giard in that "sexual maturity" is independent of somatic development. The general occurrence of birth of young in the adult stage of parthenogenetic aphids is apparently a mere case of normal coincidence, as the time required by the older parthenogenetic eggs to complete their intrauterine incubation and the time required by the mother to reach the adult stage from the date in which ovulation begins to take place are generally of almost equal duration. Presence of disturbing factors which may bring about a retardation in the metamorphosis of the mother, accompanied by a normal or more rapid development of the egg, or an unusually rapid development of the egg in a normally growing mother, will probably bring about parturition during the preadult stages of aphids.

8. As shown in Table 2, the time intervening between the extrusion of an egg in the ovarian tubule and that of the next one is from about twenty-four to about forty-eight hours. The rate of ovulation is apparently slightly more rapid during the prenatal stages and the first two nymphal instars than during the succeeding ones.

SUMMARY

1. Two eggs are extruded into each vitellarium during the embryonic stages of the mother. At birth, the mother has three eggs present in each vitellarium, the one located most anteriorly being just in the process of extrusion, and the rest, in a more or less advanced state of development. The oldest is in the early blastoderm stage.

2. A much greater percentage (about 75) of the total number of eggs which a female produces during her period of fecundity is extruded during the preadult than during the adult stage of the mother.

3. Cleavage and subsequent development of the egg proceeds soon after extrusion from the germarium.

4. Metameric segmentation of the oldest embryo within the ovary of a parthenogenetic female takes place at about the conclusion of the second instar of the mother.

5. Rotation, or orientation, of the oldest embryos occurs at about the conclusion of the third instar of the mother. At about this time, they have the nervous system, mouth parts, legs, and other parts of the body well differentiated.

6. By the time the mother reaches the adult stage, the oldest embryos have the different parts of the body completely formed and possess the general form which they finally assume when they are ready to be born, except for the relative proportion of the appendages and of the other parts of the body to one another, and for the fact that these embryos are smaller.

7. The extrusion of parthenogenetic eggs into the vitellarium of an immature mother and the subsequent intrauterine development of the former represent a true case of pædogenesis. The term unisexual entopædogenesis, as a subdivision of pædogenesis, is proposed for this peculiar method of reproduction in parthenogenetic aphids.

8. "Sexual maturity" in parthenogenetic aphids is apparently independent of somatic development. Presence of disturbing factors which upsets the balance of normal coincidence in the two processes will probably bring about parturition during the preadult stages of the mother.

OVULATION DURING THE EMBRYONIC STAGES OF THE MOTHER IN THE PARTHENOGENETIC GENERATION

An attempt was made in the present experiments to determine the length of time ovulation goes on in the embryonic mother previous to birth of the latter. It may be necessary in this connection, for the sake of clarity, to restate briefly a few of the pertinent points which I have discussed before. During the embryonic stage of the mother two eggs are extruded into each vitellarium. They proceed in their development and the older one grows to the early blastoderm stage by the time the mother is born. At about this time also, a third egg just

begins to appear from the germarium. In the twelve ovarian tubules, which is the number present in one individual, twenty-four eggs are, therefore, extruded during the prenatal stages of the mother. The embryos that are in homologous location in the ovarian tubules of an individual are of practically the same age and are apparently born in close succession to one another. From dissection of *Macrosiphum* in different instars, as shown in Table 1, the discrepancy in age between any two successive eggs in the same vitellarium is so great that the embryo situated nearer the germarium is not likely to be born before all the other embryos in the rest of the ovarian tubules which are homologus in location to the one that had preceded it are expelled. Other things being equal, the succession at birth between the first and the thirteenth young, the thirteenth and the twenty-fifth, etc., probably corresponds to the interval of time between the extrusion of the first and the extrusion of the second egg in each individual ovarian tubule; of the second and the third; and so on, respectively. Taking the third egg in the ovariole as marking the conclusion of ovulation in the embryonic stages, since it is just being extruded from the germarium at the time of birth of the mother, the intervening period to this point from the extrusion of the first egg in the same ovariole represents the period of prenatal ovulation. The only way in the present experiments by which the time intervals in the succession of any particular eggs in the same ovarian tubule may be determined is by resorting to the indirect method of observing the sequence of birth of the young which have presumably developed from those eggs. The time intervening between the extrusion of the first and that of the third egg in the same ovariole apparently corresponds to the time intervening between the birth of the first and that of the twenty-fifth young. Table 3 gives the breeding results on ten parthenogenetic adults of *Macrosiphum tanaceti* on which records have been kept of birth rate. In this table, I have given the time elapsing between the birth of the first and that of the twenty-fifth young as that of prenatal ovulation.

The figures given in Table 3 appear to have the following significance: Birth of the first young ordinarily takes place in *Macrosiphum tanaceti* from a short time after the last molt to about the third day of the adult stage of the mother. Now, the first young at about one day and fourteen hours (minimum given in Table 3) to about three days and one hour

(maximum) before birth is contained within an aphid which is either in the later part of the fourth instar or the earlier part of the adult stage (see Table 1). As is also shown in Table 1, the first young in the ovarian tubules of the mother at this stage has the body wall fully formed, and the appendages and internal organs well differentiated. It is in many respects a perfectly developed nymph, except that it has not yet attained the full size which it is to have when ready to be born. It seems justifiable to make the deduction from the foregoing facts that ovulation does not begin in the embryo until after it has undergone rotation and the definitive body walls have been formed. This condition is more or less to be expected, in view of the fact that the differentiation of the definitive internal organs, including the ovarian tubules, is not completed until after the embryo has reached this stage. Another deduction which may be made here is that ovulation does not ordinarily begin to take place in any of the parthenogenetic embryos until after the mother containing the latter has reached the later part of the fourth instar or the earlier part of the adult stage.

TABLE 3.—*Period of ovulation in parthenogenetic embryo of Macrosiphum tanaceti Linnæus prior to birth.*[a]

Culture No.	Date of birth of 1st young.		Date of birth of 25th young.		Period of prenatal ovulation.	
	1921.	a.m. p.m.	1921.	a.m. p.m.	Days.	hrs.
1	June 7	9	June 9	8	1	23
2	June 7	9	June 9	7	2	10
3	June 7	9	June 9	7	2	10
5	June 7	12	June 9	6	2	6
6	June 7	8	June 10	9	3	1
7	June 7	8	June 9	2	2	6
9	June 6	9	June 9	8	2	23
10	June 7	2	June 9	7	2	5
11	June 7	7	June 9	2	1	19
16	June 7	5	June 8	7	1	14

[a] Maximum, 3 days 1 hour; minimum, 1 day 14 hours; average, 2 days 7 hours.

SUMMARY

By correlating breeding observations with results obtained from dissection, it has been found that the period at which ovulation, that is, extrusion of egg from the germarium, begins to take place in the embryonic mother ranges from about one day and fourteen hours to about three days and one hour, the average being about two days and seven hours before the birth

of the latter. Deduction is made from the foregoing figures that ovulation does not begin to take place in the embryonic mother until after the latter has the body wall completely formed and the internal organs fully differentiated. Further, the commencement of ovulation in the embryonic mother apparently occurs coincidentally with the time that the mother bearing this embryonic mother reaches the later part of the fourth instar or the earlier part of the adult stage.

INCUBATION PERIOD OF THE PARTHENOGENETIC EGG

Like the preceding observations on the prenatal ovulation in *Macrosiphum tanaceti*, the incubation period of a parthenogenetic egg, that is, the time required from the extrusion of an individual egg from the germarium to the birth of the nymph which develops from that egg, can be approximately determined indirectly by correlating the results of breeding experiments with those on dissection of individuals of known ages. I have pointed out in the foregoing discussion that at the time the mother is born each one of the twelve ovarioles contains three eggs—two that had been laid within about one day and fourteen hours to about three days and one hour prior to birth of the mother, and a third that is beginning to be extruded at about the conclusion of the last prenatal stage. The incubation period of a parthenogenetic aphid egg, on the basis of this third egg, is calculated by recording the length of time that elapses from the birth of the mother, through her nymphal and adult stages, to the birth of her twenty-fifth young. The birth of the twenty-fifth young marks approximately the conclusion of the incubation period of the third egg in the ovariole that had presumably been the source also of the first-born young, there being normally two embryos which will have to be expelled from each of the twelve ovarioles before the third one in the first ovariole is deposited. One difficulty with the present method of calculation is that it is obviously impossible to prove that the first and the twenty-fifth nymph developed respectively from the first and the third egg in the same ovariole. Granting, however, that they each come from a different ovarian tubule, and that the correct nymph is born later than the twenty-fifth, the discrepancy in age among the third eggs in the twelve ovarioles is apparently too slight to become a serious source of error. Table 4 gives the calculated data on the incubation period of ten parthenogenetic eggs.

TABLE 4.—*Incubation period in parthenogenetic generation of Macrosiphum tanaceti Linnæus.*[a]

Culture No.	Date of birth of mother.		Date of production of 25th young.		Duration of incubation period.	
	1921.	*a.m.p.m.*	1921.	*a.m.p.m.*	*Days.*	*hrs.*
1	May 27	6	June 9	8	12	14
2	May 27	6	June 9	7	13	1
3	May 27	6	June 9	7	13	1
5	May 27	6	June 9	6	13	0
6	May 28	9	June 10	9	13	0
7	May 28	9	June 9	2	12	5
9	May 28	8	June 9	8	12	0
10	May 28	10	June 9	7	12	9
11	May 28	10	June 9	2	12	4
12	May 28	10	June 9	10	12	0

[a] Maximum, 13 days 1 hour; minimum, 12 days; average, 12 days 13 hours.

Viviparous reproduction in aphids has been referred to as a "short-cut" method of multiplication. The findings in the present experiments tend to furnish concrete evidence in support of this statement. In the amphigonous generation the entire time spent by the eggs within the ovary of the mother is used up in the accumulation and storage of food material and in the formation of the protective layers of vitelline membrane and chorion for the future embryo. The eggs are, moreover, dependent on being previously fertilized by the male spermatozoa before development can proceed. Cleavage and subsequent formation of the embryo does not begin until after the eggs are laid.

In the case of parthenogenetic aphids, on the other hand, development goes on after the egg is extruded from the germarium, even during the prenatal stage of the mother, and the complete embryonic formation takes place within the maternal ovary. The male element is not depended upon for the initiation of development. Time is further saved by using the nutriment from the body of the mother directly as it enters the egg, and the delay which would otherwise have been occasioned by the intermediate process of storage of food material, as in amphigonous eggs, is thus eliminated. On account of absence of biological data on amphigonous forms of *Macrosiphum tanaceti*, I am unable to make an estimate of the actual time saved by parthenogenetic reproduction in this species. Such comparison, however, is possible for another species, *Aphis pomi* de Geer, for which Baker and Turner (1916) furnish very complete life-history data. These authors report that the

nymphal life of the parthenogenetic form of this species embraces a period of ten to eleven days, as against sixteen to thirty-six days for a similar stage in the amphigonous female individuals. The first young in parthenogenetic mothers is born about twenty-four hours after the fourth molt of the latter, while the first egg in the amphigonous is not laid until after about two to four days have elapsed. The first egg in the amphigonous *Aphis pomi,* like the first parthenogenetic egg, is probably extruded into the vitellarium some time during the embryonic stage of the mother. I have found this to be the case in certain other species I have dissected, like *Macrosiphum tanaceti, Aphis euonomi* Fabr., and an unidentified species on *Shepherdia* (*Elæagnus*) *argentea.* Even if we suppose, however, that the period of prenatal ovulation is shorter in the amphigonous form, still the difference in time from the extrusion of the egg from the germarium to the formation of the full-grown embryo would be in favor of parthenogenetic reproduction. In the case of the first egg of the amphigonous individual, the entire time during the nymphal stage of the mother, which is sixteen to thirty-six days, is spent in accumulating the necessary substances for the proper maintenance and protection of the future embryo; the initial steps in embryonic formation do not begin until after oviposition. On the other hand, as soon as the first parthenogenetic ovum is extruded from the germarium, while the mother is still in her embryonic stage, it undergoes cleavage, forms the blastoderm, and during the ten to eleven days of the nymphal life of the mother, passes through the embryonic development; so that, in about twenty-four hours after the mother reaches maturity, the young is ready to be born as a perfect nymph, and not as an egg that has just been fertilized and is only preparing to cleave, as in amphigonous reproduction. As has been noted by Leydig (1850), Lubbock (1857), Huxley (1858), Leuckart (1858), Balbiani (1871), and other authors, there is no fundamental difference between the development of the amphigonous and that of the parthenogenetic egg, except in time of initiation of development and, of course, in the nonparticipation of the male elements in the latter process. Leuckart (1858, p. 346) gives a concise summary of the subject in the following words:

Beiderlei Gebilde sind allerdings als Zellen zu betrachten, die sich auf analoge Weise in einen Embryo entwickeln, aber in dem einen Falle, bei den Keimzellen, beginnt diese Entwicklung bereits ausserordentlich frühe, schon zu einer Zeit, in der das Material für den Aufbau des Embryo noch

lange nicht vorhanden ist, während im anderen Falle, bei den Eiern, die Entwicklung des Embryo in einer sehr viel späteren Zeit anhebt, erst dann, nachdem dieses Material vollständig herbeigeschafft und durch Ausscheidung einer festen Hülle nach aussen abgeschlossen ist.

The advantages accruing to certain groups of animals as a result of parthenogenesis, which have been pointed out by Weismann (1904, 2, p. 243) in connection with daphnids, appears to be shared by the aphids, as may be inferred from the foregoing discussion. Weismann says in part:

> Parthenogenesis effects a very considerable increase in the fertility of a species, and in this increase the reason for its introduction among natural phenomena obviously lies. By the occurrence of parthenogenesis, the number of ova produced by a particular colony of animals may be doubled, because each individual is a female, and as the multiplication increases in geometrical ratio a few parthenogenetic generations result in a number of descendants enormously in excess of those produced by bisexual reproduction.

SUMMARY

The incubation period of the third oldest egg in the ovariole, which is the egg that has been found to be in the process of extrusion from the germarium at birth of the mother, is followed indirectly by recording the time elapsing between the birth of the mother and the birth of the twenty-fifth young from this mother. The incubation period has been calculated as ranging from twelve days to thirteen days and one hour, the average being twelve days and thirteen hours.

The entire embryonic development of the parthenogenetic aphid takes place in the ovariole. A great economy in time is effected by the viviparous, parthenogenetic method of reproduction, as compared with the amphigonous, to say nothing of a greater assurance of the production of offspring in the former, on account of its independence from the male fertilizing element. Further, each individual being a female in parthenogenetic reproduction, the number of ova produced "may be doubled" and thus insure a considerable increase in the "number of descendants enormously in excess of those produced by bisexual reproduction."

THE GERMARIUM, OR TERMINAL CHAMBER OF THE OVARIOLE, AND ITS INCLUSIONS; THE FORMATION OF THE EGG FOLLICLE

The germarium of parthenogenetic *Macrosiphum* presents a subellipsoidal or subpyriform aspect, with the distal half or two-thirds usually somewhat larger in cross-sectional diameter than the proximal. In the later nymphal instars and in the adults,

it tends to assume an elongately subglobular form. At about the time of or a little after differentiation in the embryonic mother (Plate 3, fig. 20), prior to the extrusion of any of the oöcytes into the vitellarium, the germarium is obovoid, and measures from 32 to 36 micra long and 18 to 23 micra in its greatest cross-sectional diameter. The enveloping wall at the anterior and lateral portions is lined entally with a single layer of cells which is almost uniformly 1 micron in thickness throughout. The component cells average about 2 micra in length, and show no signs of very marked stretching. This layer of cells represents the epithelium of the germarium.

A small posterior portion of the germarium is bounded by a much thicker wall (Plate 3, fig. 20, *a*), which is multilaminar at this region, and of which the component cells are about one and one-half times or twice the size of those of the anterior wall. This layer of cells protrudes posteriorly into the vitellarium. This tissue gives rise to the follicular epithelium of the egg and, for this reason, I shall hereafter refer to it as the formative egg follicle. The vitellarium at this stage is about 3 to 4 micra in cross-sectional diameter.

Plate 3, fig. 22, *a*, shows a stage in the later history of the formative egg follicle. The component cells have grown considerably larger, now averaging about 3 micra in their largest expanse, and the nuclei, which are subellipsoidal, about 2.5 micra in length. I have not been able directly to observe mitosis in this region but, judging from their very considerable increase in number, the cells must have proliferated actively, and it is probably the rapidity with which cell division takes place that makes it difficult to find karyokinetic figures among them. The illustration just referred to also shows an egg in the process of extrusion from the germarium, and the formative egg follicle completely inclosing the former. There can be little doubt that the following process occurs: This thick epithelium is the matrix of the egg follicle. It divides rapidly and in this way covers the developing egg, from the posterior pole anteriorly. Finally, as growth of the formative egg follicle proceeds, its layer of cells soon closes over the anterior pole of the egg (Plate 3, fig. 23, *a*), thus completely inclosing the latter. In this manner, the egg is provided with a follicle. That part of the epithelium now bordering the anterior pole of the egg thickens considerably and replaces the original matrix. In the meantime, another egg is extruded from the vitellarium. The process of formation of the follicular epithelium of the egg by posteroanterior circum-

crescence is then repeated, each succeeding matrix of course being a direct descendant of the original formative egg follicle. As each new egg makes its appearance in the vitellarium, the older eggs are pushed, perhaps mainly by mechanical propulsion resulting from increase in pressure from the anterior region, toward the posterior portion of the tube. The follicular epithelium which incloses the egg, together with the corresponding matrix of the former, accompanies it in its downward migration.

The remnants of the matrices (Plate 3, fig. 22, *c*) persist for a time as solid, subcylindrical structures, with their component cells identical with those of the egg follicle proper. Each of these structures forms a posterior peduncle connecting one egg chamber with the contiguous one. The peduncle probably performs the same physiological functions as the egg follicle proper, which are mainly nutritive, in addition to serving as a cushion between neighboring egg chambers, and—a very important purpose—as storage tissue for the "symbionts" which, as I shall describe later, infect the egg through the posterior pole. This structure apparently degenerates later, or becomes absorbed as an integral part of the egg follicle proper during the stretching of the latter, thus ultimately setting the egg chambers free from each other.

Observations somewhat similar to the earlier phases of egg-follicle formation have been reported, in a rather cursory way, by Metschnikow (1866), and somewhat crudely represented in Plate 28, figs. 1 and 7, of his paper. He notes that—

> die am untersten Pole des Endfaches liegende Zelle sich bedeutend vergrössert, wobei sie in ein, aus dem Endfachepithel entstandenes Follikel eingeschlossen wird und hier ihre weitere Entwicklung vollzieht.

He has not followed the details of the process any further.

Figure 22 also shows a marked stretching of the epithelial lining of the anterior and lateral walls of the germarium, as a result of the increase in volume of the cellular elements inclosed in the chamber. In fig. 20, the corresponding epithelial cells in this region average about 1 micron thick and 2 micra long; but in fig. 22 they are shown to have stretched to about 7 micra in length, this increase probably being due also, partially, to growth, while their thickness has been reduced to 0.4 of a micron. The nuclei average about 6 micra in length and about 0.2 of a micron in thickness.

The cavity of the germarium is occupied by a mass of cells which are apparently potentially subglobular but which, owing

to mutual pressure, present a subpolygonal aspect. In the terminal chamber, in a young embryonic mother, such for instance as the one shown in Plate 3, fig. 20, which represents a very young ovariole of *Macrosiphum rosæ* drawn from an embryo which is three-fourths the size of a full-grown one, the cellular inclusions average about 5 micra in diameter. They are almost uniformly similar in size, shape, and structure. The surrounding cytoplasm is finely granular, with no evidence of vacuolation. The nuclei are subglobular, with a well-defined nuclear membrane and with chromatin granules sparsely distributed in the karyolymph. The nucleoli are not very distinct. I have never observed any of these cellular inclusions in mitosis, although de Baehr (1920) claims that at this stage, in "*Aphis palmæ*," "généralement un grand nombre des oogonies se divisent a méme temps (synchroniquement)."

With the subsequent growth of the ovariole, however, a stage is reached in which these cells exhibit marked differences. Plate 3, fig. 21, drawn from a full-grown embryo of *Macrosiphum rosæ* which is almost ready to be born, shows this change. All the cells are approximately equal in size, all being about 7 or 8 micra in diameter. Within the anterior two-thirds of the germarium, the cells (fig. 21, *og*) have a subglobular nucleus with a clearly defined nuclear membrane and a distinct nucleolus. Numerous chromatin granules are scattered evenly in the karyolymph. In proportion to the volume of the nucleus, there is present a large amount of cytoplasm, which stains somewhat deeply in Ehrlich's acid hæmatoxylin and which is very highly vacuolated. The cells located posteriorly (fig. 21, *oc*), which are less numerous than those at the anterior portion of the germarium, on the other hand, differ from the anteriorly situated ones in that their nuclear membranes are not very distinct. By contrast also, their cytoplasm shows very little sign of vacuolation. The latter is finely granular, stains deeply with Ehrlich's acid hæmatoxylin, and forms a relatively much thinner sheet surrounding the nucleus. There is no distinct nucleolus, and the chromatin material has gathered together centrally into a loose subglobular mass of discrete, darkly staining, irregularly shaped, elongate bodies. A clear sheet of karyolymph surrounds this mass, occupying a considerable space between the periphery of the latter and the ectal boundary of the nucleus.

This difference in cytological structure between the two groups of cells is interesting in that it has been responsible in part for

a protracted discussion among workers on aphid embryology in regard to the true nature of the cellular inclusions in the germarium of parthenogenetic aphids. There are two conflicting views, which may be briefly summarized as follows:

1. The cellular inclusions of the germarium represent only true oöcytes, there being no nurse cells present in parthenogenetic aphids. Among more recent workers, this view has been supported mainly by Stevens (1905) and by Mordwilko (1907).

2. The cellular inclusions represent two kinds of cells: nurse cells and true oöcytes. Will (1883) was an early advocate of this view. He claims that the nurse cells are pedunculated and are arranged radially around a central "rachis." The latter is connected posteriorly with a nutritive string which, according to him, serves to conduct the formative yolk substance from the nurse cells to the developing egg, as in amphigonous aphids. This author regards the pedunculated cells in the terminal chamber as egg anlagen which have lost their power of becoming eggs. One of Will's predecessors, Claus (1864), has proposed somewhat similar views, although he did not recognize any nurse cells, but did recognize glandular cells, by which he probably also meant the former. Among the more recent supporters of Will's view are Tannreuther (1907) and de Baehr (1920).

De Baehr gives a convenient summary of this phase of the subject in his paper. His criticisms against the evidence presented by the sponsors of the first view and his contention in favor of the stand he takes appear on superficial examination to be plausible, except for the fact that his arguments are weakened by the very poor figures which he used in his illustration. In justice to this author, however, it must be mentioned here that, as he states in his paper, he had lost his more carefully prepared drawings during the recent European War and that he was thereby compelled to resort to a hastily prepared and less satisfactory substitute. In his Plate 1, figs. 1, 2, and 3, in which he represents what he thought were the oöcytes and the nurse cells within the germarium, the morphological distinction and the location of these two groups of cells are not sufficiently convincing. The cellular elements which he took for oöcytes appear to me to be the cells of the formative egg follicle at the posterior portion of the terminal chamber which, in sectioned preparations, are often in the way, and may at times be so situated as to be mistaken for cellular inclusions of the germarium.

In my own work, in which I have examined numerous slides of two species, *Macrosiphum tanaceti* and *M. rosæ*, the structural

differences of the cellular inclusions which I have described above are well marked in the latter only. In *M. tanaceti,* some of the cells found at the posterior portion of the full-grown germarium show large masses of chromatin granules which are scattered in the karyolymph (Plate 3, fig. 22, *oc*). The nucleolus is absent. In these particulars, they are very suggestive of the nuclear structure of the young ovarian egg (*ov*), which is represented in the same figure as being extruded from the germarium. In other respects, however, these posteriorly located cells are very similar to those at the anterior portion of the terminal chamber (fig. 22, *og;* fig. 24), the only visible difference being that the latter are in a resting state and possess distinct nucleoli.

Adult *M. tanaceti* were dissected four days after their emergence, in order to determine the contents of the germarium at this stage. Practically all the eggs that a parthenogenetic female of this species is capable of producing will have been extruded from the germarium by this time. This conclusion appears to be supported by the fact that, in all of the females at this age that I have dissected, the germarium is adjoined posteriorly by an egg in a very advanced state of growth, often being in a later stage of blastoderm formation, while occasionally it is found to have developed as far as the beginning of the metameric segmentation of the germ band. No ovarian egg was seen in the process of extrusion, indicating that at about this period in the life of *Macrosiphum* ovulation ceases. The germarium at this time still incloses from fifteen to twenty cellular elements, with a subglobular nucleus and a distinct nucleolus. This fact shows that not all of the cellular inclusions of the germarium go into the formation of ovarian eggs.

The data I have so far obtained in my work point to the following considerations:

1. Following the differentiation of the definitive terminal chamber, or germarium, in the embryonic mother, the cellular elements inclosed within it are similar to each other in size, shape, and structure.

2. These cells increase in size and, at a later stage in development, a few that are located in the posterior portion of the germarium exhibit a marked difference in structure from the rest of the cells.

3. At the termination of the period of fecundity of the mother, that is, when the eggs cease to be extruded from the germarium, a large number of the original cellular inclusions, probably about

twice those that have been extruded as ovarian eggs, remain within the chamber.

From a correlation of the foregoing three points, it seems justifiable to offer the following interpretations: The germ cells, by the time they are first inclosed by the walls of the definitive terminal chamber, have probably developed as far as the ultimate, or secondary, oögonia. This supposition is based on the fact that, after this stage, no further oögonial division has been found to take place. They are all similar in size and in cytological characters, and are apparently in a resting condition. Later, however, after increasing considerably in volume in common with all the rest within the chamber, the cells in contact with or in the immediate neighborhood of the formative egg follicle begin to enter into a period of growth or pass into the early prophase of the first maturation (?) division, in the manner which I have described above. A similar behavior of the posterior cells has been previously reported by Stevens (1905). The cells apparently remain in this condition, without increasing any further in volume, until after extrusion into the vitellarium. The earliest manifestation of this growth or mitotic activity apparently represents the transition of the cells from ultimate oögonia into oöcytes. The latter develop successively from the oögonia, and are extruded from the germarium, one after another, until a maximum limit of about nine eggs, or a total of one hundred eight eggs in the twelve vitellaria, is reached. The maximum number is probably determined by the ability of the mother to nourish the rapidly developing embryos directly from her own body fluid,[14] for viviparous reproduction undoubtedly saps the vitality of the mother to the extreme.

Although the cells which remain behind within the vitellarium at the conclusion of the period of fecundity may, from the beginning of ovulation, have possessed a secretory function which contributes to the nourishment of the developing eggs, it would not be correct to regard them as nurse cells, sui generis, homologous to the aggregation of cells known by that name in amphigonous aphid individuals. In the latter case, the nurse cells are actually differentiated, as such, since the early stages of definitive egg formation, although these nurse cells arise from the primitive germ cells, in common with the eggs. After differentiation, however, their structural changes become so great that

[14] This idea has been suggested to me by Prof. W. E. Castle, to whom my thanks are due.

the nurse cells lose their original egg-forming potentiality. On the other hand, in the case of parthenogenetic aphids, we have within the germarium a mass of cells that are apparently similar to one another in structural characters at all times. They probably are all formative ovarian eggs, the potentiality of which is not lost through further specialization, the individuals among them that proceed in development being controlled numerically by the physiological limitations of the mother. The extra number present in the germarium probably represents a reserve supply from which additional ones may be drawn when necessary. While these supernumerary oögonia are in their resting condition, they probably produce secretions which, together with the secretion from the egg follicle, nourish the developing egg. A somewhat similar idea has been previously suggested by Mordwilko (1907). It may be mentioned in this connection that I have so far failed to find the "central rachis" of Will and the "nutritive string" in any of the material I studied, although I looked for them particularly, both in sections and in fresh preparations. None of the cellular inclusions of the germarium have been found by me to be pedunculated, either, for they are all subpolygonal and resemble each other, except that those which I have interpreted as ultimate oögonia in the anterior portion of the chamber are in a resting state, while the oöcytes at the posterior portion are either in a period of growth or in early prophase.

It should also be noted at this juncture that the old view of Claus (1864, p. 44) and of Lubbock (1857), that the eggs of parthenogenetic aphids, as well as the "nurse cells," arise as a modification of the follicular epithelium, which was adopted with slight modification by Tannreuther (1907), is no longer tenable. The ultimate oögonia are present in large numbers in the germarium since the early differentiation of the latter. The oögonia, as their origin is generally considered by workers on different groups of animals, arise, not from the follicular epithelium, but from the primitive germ cells, which are segregated some time during the earlier stages in the development of the egg.

SUMMARY AND CONCLUSIONS

1. The follicular epithelium of the egg arises from a mass of rather large cells, adjoining the posterior end of the germarium. This tissue is present in this region since the early differentiation of the definitive terminal chamber. On the basis of its subsequent history, I am referring to it as formative egg follicle.

2. The formative egg follicle acts as a matrix, which, by its posteroanterior circumcrescence, provides the developing oöcyte with follicular epithelium.

3. The portion of formative egg follicle which closes over the anterior pole of the egg proliferates, until a thick, multilaminar layer is formed. This new layer replaces the original matrix, and the process of formation of the egg follicle around each succeeding egg is repeated.

4. The remnants of the matrices of the egg follicle persist as a posterior peduncle connecting the egg chamber with the contiguous one. The peduncle persists for a time; then it apparently ultimately degenerates, or is absorbed by the egg follicle proper during the stretching of the latter, thus finally leaving the neighboring egg chambers free from each other.

5. The peduncle probably supplies the egg with nutritive material, in common with the egg follicle proper, in addition to serving as a cushion between every two egg chambers and as storage tissue for the "symbionts" for the future infection of the egg.

6. After the definitive differentiation of the germarium, the cellular elements inclosed within its chamber apparently represent ultimate, or secondary, oögonia.

7. The oögonia increase in size within the germarium and stretch considerably the enveloping wall of the latter.

8. At a later stage in development, the oögonia which are in contact with or in the immediate vicinity of the formative egg follicle enter a period of growth or pass into the early prophase of the first maturation (?) division. The beginning of growth or of early mitotic activity marks the transition of the cellular elements within the germarium from oögonia into oöcytes.

9. The oöcytes remain without proceeding further in their karyokinetic processes until some time after extrusion into the vitellarium.

10. The cells present in the germarium are apparently not differentiated into definitive nurse cells and definitive oöcytes, as certain authors have claimed, but are all unmodified, potential ovarian eggs.

11. The oögonia which develop into ovarian eggs are apparently numerically limited by the capacity of the mother directly to furnish nourishment from her body fluid.

12. The resting oögonia may, while in that state, have a secretory function, contributing to the nourishment of the developing egg.

13. A large number of supernumerary oögonia remain in the germarium at the conclusion of the period of fecundity of the mother. They probably represent a reserve supply, serving to insure the production of the largest possible number of offspring.

14. The oöcytes are not modifications of the follicular epithelium, as has been claimed by certain authors. They arise from the secondary oögonia which are already present in the germarium and which, as I shall attempt to show later, in turn, originate from the primitive germ cells.

DEVELOPMENT OF THE PARTHENOGENETIC EGG

MATURATION AND CLEAVAGE STAGES, WITH REMARKS ON EARLY INFECTION OF THE EGG BY THE "SYMBIONTS"

The early phases of oögenesis in parthenogenetic aphids have been the subject of extensive investigation by earlier workers, principally by Will (1883), Blochmann (1887), Petrunkevitch (1903), Stschelkanovzew (1904), Stevens (1905), Tannreuther (1907), and de Baehr (1920, and also earlier papers by the same author). As Blochmann (1887) has first pointed out in connection with *Forda formicaria,* the parthenogenetic eggs of aphids extrude only one polar body, similar to the condition which Weismann and Ishikawa (1888) have observed subsequently in the parthenogenetic eggs of certain rotifers and crustaceans. The zygogenetic ones, on the other hand, extrude two, in the same manner as the eggs of other amphigonous animals. Further, he discovered that the parthenogenetic eggs of aphids retain the somatic number of chromosomes, in a manner which has been variously explained. In regard to this point, there are two principal views, which are essentially the same, except in the details: (*a*) That of Stevens (1905), who claims that in the parthenogenetic aphids there is but "one maturation division without reduction," and (*b*) that of de Baehr (1920), whose contention is that—

> les oöcytes de l'*Aphis palmæ* montrent encore à la prophase de maturation * * * des tendances hétérotypiques bien nettes, et c'est seulement à la diacinese, par la dissociation des gemini en leur composants (les chromosomes simples), qu'ils retournent a la mitose somatique.

This question, which needs further cytological investigation, falls outside the scope of the present paper.

The development of the oöcyte from the oögonium has been described above. As I have already shown, during the posteroanterior circumcrescence of the formative egg follicle around the oöcyte, the latter retains its direct communication with the

terminal chamber for a time, before the complete closure occurs. In the meantime, the oöcyte increases considerably in size, so that by the time it is completely inclosed by the egg follicle (Plate 3, fig. 23, *ov*), it has grown to several times its original size. The cytoplasmic layer is finely granular, and stains rather deeply in Ehrlich's acid hæmatoxylin. The nucleus, which is located centrally in the ovarian egg,[15] has also grown considerably larger and is apparently vesicular, with a few loosely joined chromatin granules arranged in a subcentral position. The increase in size of the ovarian egg is probably partly due to growth of the cell itself and partly to deposition in the cytoplasmic mass of reserve food material, which, as has been suggested by Wheeler (1889) in connection with *Blattella germanica,* is "secreted by the protoplasm of the epithelial cells, not as yolk, but as substances taken up by the growing ovule." A part of this protovitelline material, in aphids, probably originates also from secretion of the cellular inclusions of the germarium during the time that the formative egg follicle has not yet completely inclosed the ovarian egg.

Before extrusion of the polar body, the ovarian egg of *Macrosiphum tanaceti* is subellipsoidal, and is longer anteroposteriorly than laterally. It attains a maximum length of about 25 micra and cross-sectional diameter of about 17 micra. The subglobular nucleus is about 9 micra in diameter, with a few scattered chromatin granules which are not sharply defined in outline. An egg in that stage is shown in Plate 3, fig. 25. The follicular epithelium, which is not shown in the figure, is closely applied around the egg. It is unilaminar and measures about 1 micron in thickness, except at the anterior and posterior poles, where it is stratified, with a corresponding measurement of about 3 micra.

Soon the nuclear membrane of the ovarian egg disappears, and the nucleus undergoes mitosis. At the same time, numerous vacuoles which have concentrated together and formed a cytoplasmic network appear posteriorly and laterally near the nucleus, leaving a thick, irregularly shaped cytoplasmic mass in which mitosis takes place. A thick, peripheral cytoplasmic layer, which apparently represents the early formation of the so-called Keimhautblastem (Weismann, 1864, p. 5), periplasm, or perivitellus (Korschelt and Heider, 1899, p. 262), bounds the cytoplasmic network ectally. As has been suggested before by Blochmann (1887) and by Stevens (1905), the network ap-

[15] The oöcyte at this stage of growth is usually termed "ovarian egg." (Sharp, 1921, p. 221.)

parently contains yolk, which is not easily distinguishable in either the fresh material or in sections because of its scarcity, the small size of the globules, and also the fact that it is probably dissolved out by the histological reagents used.

The nucleus at this stage is eccentrically located, toward the anterior pole of the egg. Plate 3, fig. 26, shows the nucleus in late anaphase, in that position. The cleavage plane is subperpendicular to the longitudinal axis of the egg. This condition apparently represents the extrusion of the only polar body. At the conclusion of telophase, the daughter nucleus which is located posteriorly becomes entirely isolated, together with a layer of enveloping cytoplasmic mass, from the periplasm through the anterior extension of the cytoplasmic network. The slender cytoplasmic strands of the network serve as connecting bridges between the centrally located nucleus and the periplasm. The polar body, which remains within the periplasmic mass, probably disintegrates after a short time. I have never had occasion to observe the polar body in any of the parthenogenetic aphid eggs I have studied, and my failure to do so is probably due to this rapid disorganization.

Plate 3, fig. 27, shows an egg after the extrusion of the polar body has taken place. The egg is irregularly subglobular, greater in lateral than in anteroposterior diameter, and measures about 30 by 24 micra. The nucleus is more uniformly subglobular, measuring about 9 micra in diameter, and is somewhat eccentrically located in the egg. The chromatic network is very open, so that the nucleus appears vesicular, with a few small, faintly staining chromatin granules. There is an enveloping thin, irregular sheet of finely reticular cytoplasm which connects radially with the strands of the surrounding cytoplasmic network. The periplasm covers the entire periphery of the egg, and is of variable thickness.

In the first cleavage, the poles of the mitotic spindle are directed anteroposteriorly, almost in the same direction as the longitudinal median axis of the egg. The egg at this stage (Plate 4, fig. 28) is subglobular, with a diameter of about 30 micra. The cleaving nucleus is somewhat eccentric in its location in the egg, and is separated from the periplasm by the intervening cytoplasmic network, as in the previous stage. The periplasm continues to cover the entire periphery of the egg.

By the conclusion of the first cleavage, the egg has elongated anteroposteriorly into a narrowly subellipsoidal form, and the two daughter nuclei are found each toward one of the poles (Plate

4, fig. 29). The egg at this stage is about 43 micra long and 23 micra in its greatest diameter. The cleavage nuclei are subglobular and measure about 8 to 9 micra in diameter. A few minute chromatin granules are sparsely scattered in the faintly staining karyolymph. The periplasm still persists at the entire periphery of the egg. The follicular epithelium is detached from all the sides of the egg, except at the posterior pole, with which it is still closely connected.

After this stage, all the daughter nuclei appear to undergo mitosis, simultaneously at first; later, however, they do not all divide at the same time.

It is interesting to consider at this juncture the marked changes in the shape of the egg during the earlier cleavage stages. We have noted that prior to the extrusion of the polar body the egg presents an abruptly subellipsoidal aspect. This shape persists until after the completion of the process, the egg then exhibiting only an increase in size. It then assumes a subglobular form, until first cleavage, when it begins to elongate again anteroposteriorly into a subellipsoidal form, this time more narrowly so. I take these abrupt changes in shape to be due to a rapid progressive increase in the volume of the egg, as a result of further vacuolation in the cytoplasmic mass and consequent increase in size of the cytoplasmic network. The force of expansion within the egg accompanying such active vacuolation changes from one direction to another, being alternately greater anteroposteriorly than laterally, and vice versa.

The periplasm persists at the entire periphery of the egg even to the two-nuclei stage. Later, however, a direct communication is established between the cavity occupied by the cytoplasmic network in the interior of the egg and the follicular epithelium by the disappearance of a portion of the periplasm from the posterior pole. Plate 4, fig. 30, represents an egg with at least four cleavage nuclei, in anaphase. At the posterior pole there is very marked vacuolation and thinning of a small portion of the periplasmic sheet. This condition apparently represents the initial steps in the removal of the cytoplasmic layer from this region.

In the same figure, the migration of the cleavage nuclei into the periplasm is illustrated. Upon coming in close proximity to the latter, the cytoplasmic layer enveloping the cleavage nucleus coalesces with the periplasm. The substance in the two masses being apparently identical, the cytoplasmic envelope of the cleavage nucleus becomes absorbed as an integral part of the peri-

plasm. The nucleus is then drawn more deeply into the thick cytoplasmic sheet, probably by surface tension in the latter, and in this way becomes embedded in the periplasm. The relation of the cleavage nuclei to the periplasm at this early stage has apparently no morphological significance, for there is evidently a tendency to return to the cytoplasmic network in the interior of the egg, at least in the case of some of the nuclei.

Earlier workers on aphid embryology have ascribed a great deal of importance to the question of whether in these earlier cleavage stages some of the nuclei lag behind and become predestined as "vitellophags," or whether all of the nuclei become embedded ultimately in the periplasm, the "vitellophags" arising as a result of later immigration from the periphery. Witlaczil (1884) claims to have observed the first condition, and he has been supported in this view by Stevens (1905) and by Tannreuther (1907). Will (1883), on the other hand, takes the opposite view, maintaining that all of the cleavage nuclei migrate to the periplasm. In a more recent paper Hirschler (1912), after giving a concise review of the subject, justly expresses his disagreement with all of his predecessors. I am quoting this author at length on account of the bearing of his remarks on the present question:

> Ich möchte nämlich den gegen die Eimitte vorgerückten Kern für einen Abkömmling des central im Ei gelegenen Furchungskernes des zweizelligen Stadiums ansehen, wobei der zweite an die Peripherie gerückt ist. Es scheint mir nun im allgemeinen berechtigt, angesichts dessen, das in manchen Fällen einer der zwei Kerne central gelegen und dass im vierzelligen Stadium wiederum oft ein Kern mehr centralwärts gelagert ist, anzunehmen, dass bei *Rhopalosiphum* nicht immer alle Kerne an die Peripherie gelangen, sondern dass auch hier, wie bei den meisten Insekten, Furchungsproducte im Eiinnern zurückbleiben. Merkwürdig könnte hier vielleicht dies erscheinen, dass bei ein und derselben Art die Furchungskerne ein verschiedenes Verhalten aufweisen, dem gegenüber wäre zu erwähnen, dass die dotterarmen, kleinen Aphideneier eben zur Feststellung dieser Varianten sich vorzüglich eignen, während dieselbe, bei den dotterreichen, grossen Insekteneiern erheblich erschwert wird, wodurch auch die bezüglichen Vorgänge noch immer nicht genügend in ihren Einzelheiten bekannt sind. Anderseits wäre auch hervorzuheben, dass die Kleinheit der Aphideneier das bestehen solcher Varianten gegünstigt, denn auch ganz geringe Kernverschiebungen, die in den grossen Insekteneiern oft nur unter geordnete Differenzen hervorbringen könnten, verursachen bei den ersteren schon ziemlich grosse Entwicklungsvarianten. Angesichts dessen nun, dass die Entwicklungsvarianten durch die Kleinheit der Eier begünstigt, letztere aber durch die Dotterarmut, die wiederum höchst wahrscheinlich mit der sekundär erworbenen Viviparität im Zusammenhang steht, verursacht wird, möchte ich ihnen keinen grösseren morphologischen Wert zuschreiben.

My own observations are in accord with those of Hirschler in that migration of the cleavage nuclei into the periplasm apparently occurs soon after the first cleavage. I have never observed in my material any instance in which, after the first cleavage, one of the daughter nuclei, as he claims, migrates to the periphery, while the other lags behind within the cytoplasmic network. I did find, however, that after the second cleavage (Plate 4, fig. 30) three of the daughter nuclei are embedded in the periplasm while one is isolated by the cytoplasmic network. His contention that the two opposite points taken up by the earlier authors have no morphological value seems to be tenable, on the basis of the reasons he has given. Following this question further, I may add that, during the earlier stages in the development of the egg, there is a period in which an active shifting and reshifting of the cleavage nuclei from one part of the egg to another apparently takes place. Whether this movement of the cleavage nuclei is due to their own activity, in which the pseudopodialike projections from the surface of their enveloping cytoplasm play a part, or whether they are transported mechanically by the cytoplasmic stream of the egg has not been satisfactorily determined. As a result of these movements, certain nuclei come in contact with the ental surface of the periplasm and become embedded in the latter, in the manner I have described. During the earlier cleavage stages the latter process is not necessarily an expression of the initial steps in the organization of the blastoderm, for there are many cases in which the nuclei soon dissociate from the periplasmic layer and return to the yolk mass. It seems to be more reasonable to consider the nuclei in these earlier stages, as represented in Plate 4, figs. 29, 30, 31, and 32, as plastic units, all of which are potential blastomeres—conditional upon their finally remaining in the periplasm—but the ultimate destiny of none of which is definitely predetermined. During these plastic stages, all the nuclei in the developing egg, at one time or another, apparently have some relation with the function of converting the protovitelline material in the cytoplasm into yolk. The formation of vacuoles in the cytoplasm is apparently caused by the deposition of yolk in small separate masses. That the activity of the cleavage nuclei is apparently responsible for this process is indicated by the suspicious relation of the appearance of the earliest cytoplasmic network in the egg with the first mitotic phases accompanying the extrusion of the polar body.

Soon, however, a state of equilibrium is reached when the apportionment of the cleavage nuclei into two definitive sets takes place, as follows: (*a*) The nuclei which ultimately remain in the cytoplasmic network, and continue the earlier common function of all the cleavage nuclei—the elaboration of food material. They are in this respect homologous to the "vitellophags" of other insect eggs. However, they represent the progenitors of the cells which ultimately compose the "mycetom." For this reason, I shall refer to these yolk nuclei of aphids as "mycetoblasts." (*b*) The nuclei which definitely remain embedded in the periplasm and ultimately give rise to blastomeres. Plate 4, figs. 33, 34, and 35, show three consecutive stages during the primary differentiation, which I have spoken of above. In fig. 33 the nuclei which have migrated to the periphery (*fb*) apparently remain embedded in the periplasm. Two of the "mycetoblasts" (*mb*) are shown. In fig. 34 the formative blastomeres have increased considerably in number, by tangential division, so that they are arranged much more closely to one another than in fig. 34. This closeness of the nuclei may be due also to an anteroposterior compression which results from the noticeable shortening of the egg at this stage. The "mycetoblasts" are apparently arranged sublinearly along the longitudinal axis of the egg. Figure 35 shows some of the nuclei in the periplasm and two of the "mycetoblasts" in mitosis. As may be judged from the figure, division does not occur synchronously in all the nuclei.

An earlier part of the present account has left the periplasm still covering the entire periphery of the egg. Mention has already been made of the fact that in Plate 4, fig. 30, which represents an egg undergoing third cleavage, the periplasm at the posterior pole has been reduced to a comparatively much thinner sheet than the rest and shows signs of active vacuolation. This condition apparently represents the initial steps in the formation of the opening of the periplasm at the posterior pole of the egg. The process of removal of the cytoplasmic layer from this region apparently occurs rather rapidly, the vacuoles soon expanding, and reducing the cytoplasmic sheet into an open network, in common with the rest in the interior of the egg. In this way the opening of the posterior pole is established.

It should also be noted that, as shown in Plate 5, fig. 29, while the egg is in the two-nuclei stage and the posterior open-

ing of the periplasm has not yet formed, the follicular epithelium becomes detached from around the entire periphery of the egg, except at the posterior pole, where it remains intimately in contact with the surface of the egg. The follicular epithelium at this region exhibits considerable thickening in a portion contiguous to the posterior peduncle of the egg chamber (not shown in figure).[16] This thickening indicates that, even before visible signs of vacuolation appear, diffusion of yolk mass from the interior of the egg to the egg follicle probably occurs.

The opening of the periplasmic wall at the posterior portion of the parthenogenetic aphid egg has been known for a long time. Will (1889) has described its presence, and given a detailed account of its significance. He has reported the incursion, through this opening, of "todte Nahrungsmasse" which originates from the follicular epithelium and gives rise to the "pseudovitellus." He refutes, correctly, the older views of Metschnikow (1866) and of Witlaczil (1884), both of whom have claimed that the "pseudovitellus" arises directly from the follicular epithelium, by migration of nuclei from the latter into the interior of the egg. Will did not even suspect the relation of the "vitellophags" ("mycetoblasts") to the subsequent history of the "mycetom," for he is sponsor to the idea that they enter into the formation of the mid-intestine. The incursion of the "symbionts" from or, rather, through the follicular epithelium, in coccids as well as in aphids, has been reported by Šulc (1910), Pierantoni (1910, 1914), Buchner (1912, 1921), Shinji (1919), and others. The most generally admitted view is that the follicular epithelium has something to do with the introduction of the "organisms" into the developing egg, although no very definite theory in regard to the actual source of infection has been proposed, except the one by Will, which I have already mentioned; that of Šulc (1910), who reports that in

Aphis amenticola Kaltenbach (?) habe ich an den unteren Polen der ganz jungen Eiern, wo vom Pseudovitellus noch keine Spur war, äusserlich unsere Hefe frei kleben gesehen; sie mussten also auf welche Art die Mycetocyten, auf welche sie sonst angewiesen sind verlassen haben und schickten sich zum Eindringen in das Ei zwischen den interzellulären Räumen der Zellen des Eifaches,

[16] A somewhat similar condition has been noted previously by Balbiani, as is evident from one of his figures. See Henneguy (1904, p. 405, fig. 395, A, f).

which observations have not been confirmed by subsequent investigators, and with which the results of my work also disagree;[17] and that of Buchner (1921), in which he or, rather, one of his students, claims to have seen a cell from the "symbiotic" organ of the mother in contact with the ovariole. Through an elaborate entrance in the follicular epithelium, guarded by some five nuclei, in a manner somewhat similar to that previously described by Stevens (1905), Buchner has made the "mycetocyte" pour into the egg its cell-full of "symbionts." Since the publication of Buchner's book, I have reëxamined my slides of *Macrosiphum tanaceti* for confirmation of this report. I have so far failed to find such an arrangment, and probably never shall find it. It does not seem probable that the mother would expend her "symbiotic" cells, one after another, to supply her many young which, as I have stated before, reach a total of at least one hundred eight, and have enough to supply all of them, to say nothing of the part of the organ that she apparently requires for the use of her own body. I have dissected numerous aphids in both the nymphal and the adult stages, and found that the number of cells composing the "symbiotic" organ ranges from about sixty to about seventy only; I have never found a higher figure. Flögel (1905) has discovered that, after birth of the aphid, the "mycetom" cells no longer divide but merely increase in size. My own observations have confirmed Flögel's results. This condition means that, from this limited supply at her disposal, at least in the species I have worked with, the parthenogenetic aphid mother could not have provided for all her embryos, each requiring one "mycetom" cell, if Buchner's contention were true.[18]

[17] It is highly probable that the "yeasts" which he has found free at the posterior pole of the young egg arise from infection from the swollen portion of the egg follicle. As I have described elsewhere in the present paper, the "organisms," lodged in the egg follicle, apparently begin to become activated by the egg yolk which diffuses to the latter even before vacuolation in the periplasmic layer at the posterior pole of the egg becomes evident. The rapid multiplication of the "symbionts" resulting from this stimulus causes an incursion of these "organisms" into the egg.

[18] There are, in addition, the views of Metschnikow, of Balbiani, and of other older authors, which in the light of our present knowledge of the subject are obviously unacceptable.

Will's earlier view, that the mass of granular material which enters in the formation of the "pseudovitellus" originates from the follicular epithelium, appears to be more acceptable. His contention that the granular mass is "todte Nahrungsmasse," of course, no longer holds. Emeis (1915), working on coccids, has reported similar behavior of the "microörganisms" in this group of insects. In my own work there is, it seems to me, sufficient evidence to justify the statement that "symbionts" are present also in the egg follicle of aphids.

Some time before any sign of vacuolation appears in the periplasm at the posterior pole of the egg, the part of the follicular epithelium which is closely applied to this region shows considerable thickening, which I have noted above. Upon completion of the opening of the periplasm, the swelling of the contiguous follicular epithelium becomes much more pronounced (Plate 4, fig. 32). In fresh preparation, I have observed within the meshes of the cytoplasmic network immediately adjacent to the follicular epithelium a diffuse greenish mass of granules which apparently enter the opening of the periplasm from the follicular epithelium. This migrating mass, as will be seen later from its subsequent history, represents a mass of "symbiotic organisms."[19] The "organisms" are apparently present in the follicular epithelium, mainly in the posterior peduncle, as I have stated before. They probably are in a dormant condition. When the egg yolk is made available to them through the disappearance of the intervening periplasmic layer from the posterior pole of the egg, they apparently become activated, causing them to multiply very rapidly. This rapid multiplication results in a swelling of the follicular tissue, which grows considerably larger during the later developmental stages of the egg, as may be judged from Plate 7, fig. 45, *fe*. The posterior swelling appears as a subhemispherical protuberance, which is closely applied against the opening of the periplasm and is separated from the base of the posterior peduncle only by a shallow depression. Such an arrangement is much more readily observable in whole mounts of ovarioles. The appearance of a separate swelling in the follicular epithelium suggests that the source of infection is not directly from the posterior peduncle, but from the contiguous cells in the follicular epithelium proper.

[19] In all my figures, with the exception of those in Plates 1 and 2, the "symbionts" are represented by red stippling.

The peduncle, with its large store of "organisms," probably acts as a dispenser, which continually supplies them to the egg follicle proper, since the latter, on account of its relatively much thinner wall, harbors only a very limited number of "symbionts."

The cells of the egg follicle apparently rupture and cause an incursion of the "symbionts" into the egg, these "organisms" perhaps being attracted by the egg yolk. The greenish color, which is also present as minute granular, apparently chlorophylloid, matter [20] in the "organisms" within the definitive mycetocytes of the older embryos and subsequent stages, is presumably a manifestation of metabolic activity.. Hence, no color is noticeable in the "microörganisms" which are probably dormant in the follicular epithelium, before the latter comes in contact with the egg yolk.

After incursion of the "symbionts" into the interior of the egg, they remain aggregated within the posterior third of the latter, in a more or less diffuse mass. The "mycetoblasts," which are arranged sublinearly along the longitudinal median axis of the egg, now tend to collect within the "symbiotic" fluid, so that, as shown in Plate 5, fig. 36, *mb*, they crowd together into the posterior half of the cavity of the egg, a few of them even migrating far outside the opening of the periplasm, toward the swollen follicular epithelium, where the mass of "symbionts" is presumably more concentrated. The proximity of some of the migrating "mycetoblasts" to the egg follicle is probably responsible for the erroneous interpretation by some of the earlier workers, and comparatively more recently by Tannreuther (1907), that the "pseudovitellus" arises by direct incursion of cells from the egg follicle.

The relation of the "vitellophags" to the "symbiotic" bodies was suggested years ago by Wheeler (1893). He regards them as "considerably specialized" nuclei, with "limited function and mobility," and "suspicious relations to the bacteria-like corpuscles of Blochmann."

My own observations agree in a general way with Pierantoni's (1914) account in connection with *Icerya purchasi* Maskell (Coccidæ). This author gives the following summary of his findings:

Durante la formazione del blastoderma si costituiscono anche le cellule proprie dell'organo simbiotico * * * [il quale] hanno origine da blasto-

[20] The coloring matter of the "symbionts" disappears very rapidly in alcoholic media.

meri interni che vanno ad involgere la massa polare, e che hanno valore e costituzione simile alle cellule vitelline.

The attraction of the "mycetoblasts" to the mass of "symbionts" appears to be analogous to phagocytosis. This fact suggests that the granular bodies coming into the egg from the follicular epithelium probably represent foreign protein. Such a condition very strongly supports the idea that these granules are really not by-products of the insect's own metabolism, but apparently extraneous organisms.

There is one other interesting fact in connection with the cleavage stages. Shortly before differentiation of the definitive blastomeres, a much more pronounced mitotic activity occurs among the nuclei in the periplasm at the anterior pole of the egg than in other regions. As a result of this division, a number of small nuclei (Plate 5, fig. 36, *b*) is extruded entally. These secondarily migrating nuclei are only about one-half the size of the "mycetoblasts." On account of their diminutive size, they have been mistaken for degenerating bodies. Witlaczil (1884) has erroneously interpreted them as disintegrating "vitellophags," and this view has been adopted by later workers, notably by Buchner (1921) and his student. As I have already shown, the "vitellophags" in aphids, or what I have called the "mycetoblasts," enter ultimately in the formation of the "mycetom." The smaller nuclei could hardly be considered as degenerating, for many of them are found in mitosis. An attempt is being made in a later discussion to trace their subsequent history.

SUMMARY AND CONCLUSIONS

1. By the time the egg follicle closes over the anterior pole of the ovarian egg (oöcyte during growth period) the latter has increased to several times its original size, partly by growth of the cell itself and partly through accumulation in its cytoplasm of reserve food material.

2. The reserve food material probably arises from secretions of the egg follicle, as well as from those of the cellular inclusions of the germarium.

3. Maturation is completed after the egg is completely inclosed by the egg follicle. The polar body is extruded into the periplasm at the anterior pole of the egg. The cytoplasmic network, which presumably contains yolk globules in sparse amounts within its meshes, begins to appear at the same time. The formation of this network is apparently correlated with the

activity of the nucleus, which fact indicates that the latter probably plays an important part in the elaboration of protovitelline substance into yolk.

4. Soon after extrusion, the polar body apparently degenerates very rapidly in the periplasm.

5. At the conclusion of the polar-body extrusion, the other daughter nucleus becomes surrounded with cytoplasmic network, completely isolating it from the periplasm, except for the connecting cytoplasmic strands.

6. Mitosis occurs simultaneously during the first few cleavage stages; but this regularity soon ceases.

7. Abrupt changes in the shape of the egg occur, from subellipsoidal before and during the extrusion of the polar body, to subglobular-discoidal before first cleavage, to subglobular during first-cleavage mitosis, and to narrowly subellipsoidal after first cleavage. After the last-named stage, the egg remains more or less subellipsoidal. The abrupt changes taking place during the earlier stages apparently result from an active formation of additional vacuoles, which join the cytoplasmic network. The force of expansion in the formation of these vacuoles is probably exerted with greater power alternately, in one direction first and then in another within the egg.

8. During the earlier stages in the development of the egg, the cleavage nuclei are apparently plastic units, the ultimate destiny of none of which is definitely predetermined. There seems to be a continuous shifting and reshifting of the cleavage nuclei at this stage, and whatever position they may then assume with respect to the periplasm and the central yolk mass is not necessarily an expression of initial steps in the subsequent organization of the egg. A return of the cleavage nuclei into the yolk at this time, after having become temporarily embedded in the periplasm, has presumably no morphological significance.

9. During these earlier stages, all the cleavage nuclei, at one time or another, act as vitellophags. Each is apparently a potential blastomere, conditional upon its ultimately remaining imbedded in the periplasm.

10. A state of equilibrium is soon reached when the apportionment of the cleavage nuclei into definitive formative blastomeres and definitive vitellophags takes place.

11. The vitellophags show a marked attraction to the mass of "symbiotic organisms" which invades the egg through the

posterior pole. On account of their ultimate destiny as formative "mycetom," I have adopted the term "mycetoblasts," in place of "vitellophags."

12. The affinity of the mycetoblasts to the mass of "symbionts" is very suggestive of the behavior of leucocytes in the presence of foreign protein (phagocytosis), indicating thus that these supposedly extraneous "microörganisms" are probably really such, and not by-products of the insect's own metabolism.

13. The periplasm at first forms a continuous layer at the entire periphery of the egg. Soon an opening appears at the posterior pole of the latter through the periplasmic wall.

14. The opening through the periplasmic layer arises through active vacuolation of its cytoplasmic mass, resulting in the formation of a network which becomes continuous with the similar structure in the interior of the egg.

15. At about the second-cleavage stage, the follicular epithelium becomes detached from the surface of the egg, except at the posterior pole, with which intimate contact is retained.

16. The follicular epithelium apparently harbors "symbiotic organisms," particularly in the posterior peduncle of the egg chamber, which I have previously described.

17. The formation of the opening through the periplasmic layer is evidently a special provision for the entrance of the "symbionts" into the egg.

18. The periplasmic opening arises at the region where the egg follicle remains in intimate contact with the posterior portion of the egg. This fact suggests that the opening is probably induced by secretions from the "symbionts" in the follicular epithelium.

19. The egg follicle swells considerably at the point of contact with the egg, especially when the vacuolation of the periplasmic wall begins to become noticeable.

20. The swelling lies contiguous to the posterior peduncle of the egg chamber. The latter probably acts as a dispenser of "microörganisms" to the egg follicle which, on account of its relatively thinner wall, harbors a limited supply.

21. The swelling presumably results from the activating effect of the egg yolk on contact with the "symbionts," when the periplasmic barrier is removed, resulting in a stimulation of the "organisms" to multiply with increased rapidity.

22. The mass of "symbiotic organisms" presents a greenish hue, owing to its apparently chlorophylloid, intracellular, gran-

ular inclusions. The appearance of these granules is apparently a manifestation of active metabolic processes. No green color is evident in the "microörganisms" which are presumably dormant in the follicular epithelium before this comes in contact with the egg yolk.

23. Shortly before the differentiation of the blastoderm, a few nuclei, somewhat smaller than the "mycetoblasts," migrate from the periplasm at the anterior pole of the egg into the egg cavity. These small nuclei divide actively by karyokinesis and are, therefore, not degenerating bodies.

BLASTODERMAL DIFFERENTIATION AND SUBSEQUENT STAGES, PRIOR TO METAMERIC SEGMENTATION OF THE GERM BAND

At about the time of differentiation of the blastoderm, the egg (Plate 5, figs. 36, 37) is subellipsoidal and measures about 60 micra in length and 30 micra in greatest cross-sectional diameter. The periplasm remains in the same condition as that described in the later cleavage stages: it forms a continuous dense layer at the entire periphery of the egg, except at the posterior pole, where an opening allows free communication between the part of the follicular epithelium adjoining that region and the interior cavity of the egg. The periplasmic layer apparently foreshadows the future position and extent of formation of the blastodermal wall.

The cleavage nuclei embedded in the periplasm are arranged much more closely together, on account of their greatly increased number. They are subglobular, and measure about 5 micra in diameter. A few deeply staining, large, irregular chromatin granules are present in the clear karyolymph. Mitotic figures are found, especially at the anterior pole of the egg.

The "mycetoblasts" are still in progress in their migration toward the posterior half of the egg, where they are apparently attracted by the inflowing mass of "symbionts." I judge, from their relatively larger number at a subsequent stage, that these nuclei must divide rather actively at about this time, although I have not found mitotic figures among them in the material I have on hand.

The small anterior nuclei, which I have described before as resulting from a secondary migration from the periplasm into the anterior portion of the egg cavity,[21] have also increased

[21] These nuclei will be referred to hereafter as "small anterior nuclei." No definite name can be assigned them, on account of their doubtful ultimate destiny in the later developmental stages of the embryo.

markedly in number, although they never become so numerous as the "mycetoblasts." Figure 36 shows the characteristic relative position which the "mycetoblasts" and the small anterior nuclei assume at this time. Figure 37 represents an apparently aberrant case, in which the "mycetoblasts," of which only three of the anterior ones are shown, are slow in migrating toward the posterior portion of the egg; they are still arranged in a sublinear position, as I have described in some of the earlier cleavage stages. The small anterior nuclei, which are present in the other sections of the series, are not in evidence in this particular one. The figure is intended primarily to show the manner in which the blastomeres are formed.

The differentiation of the definitive cell territories of the blastoderm occurs by a simultaneous constriction of the periplasmic layer, between the nuclei. Such a condition represents a much simpler case than the relatively more complicated process described by Weismann (1882) in *Chironomus,* by Blochmann (1887) in *Calliphora* (= *Musca*) *vomitoria* Linn., and by Heider (1889) in *Hydrophilus.* In these groups, there occurs in the periplasmic layer, between the nuclei, a surface furrowing which progresses entally until the complete lateral cell boundaries are marked out. In the meantime, a secondary "Keimhautblastem," which is devoid of nuclei, appears beneath the first. The furrows mentioned above continue through to the ental surface of the underlying layer, which results in the latter being added to the height of the blastomeres.

Some of the earlier blastodermal stages in parthenogenetic *Macrosiphum tanaceti* are shown in Plate 5, figs. 38 and 39, and Plate 6, figs. 40 and 41. The egg shown in fig. 38 represents a stage in which the blastoderm has just been completed. It is about 60 micra long and 30 micra in greatest cross-sectional diameter. These measurements are practically identical with those of the latest cleavage stages described above, a condition which points to the fact that the transition from a syncitial stage of the nuclei in the periplasm to the differentiation of the definitive blastomeres is accompanied by no appreciable increase in the size of the egg. At this relatively early stage, the unilaminar structure of the blastodermal wall is still in evidence. The wall covers the egg at the regions where the periplasm has been located previously, and is somewhat thicker at the anterior pole, whence it gradually attenuates posteriorly to the rim of

the opening, which is now the "blastopore."[22] The blastomeres are relatively large, somewhat columnar cells, showing only slight signs of lateral compression, as a result of mutual pressure. The nuclei are subellipsoidal or subglobular, measuring from about 5 to about 7 micra in diameter. One noteworthy point relative to the blastodermal wall at this early stage is that it extends only to about three-fourths the total length of the egg chamber (compartment inclosed by the egg follicle), leaving the posterior one-fourth free.

The "mycetoblasts" are about 6 to 7 micra in diameter. Their aggregation occupies more than one-half the blastocœle, and extends posteriorly outside the blastopore to the portion of the egg chamber which is free from blastodermal wall. The mass of "symbionts" which has come to occupy the meshes of the cytoplasmic network among the "mycetoblasts," where the "organisms" gradually appropriate the egg yolk, is still diffuse. The strands of the network join the "mycetoblasts," so that these nuclei are not actually free from each other, but are in syncytium.

An inflow of the "symbionts" from the follicular epithelium probably still takes place, although in much more limited quan-

[22] The term "blastopore" is used in this connection merely to indicate the opening of the blastocœle. Will (1889) has employed the same term in connection with his contention that at this stage in aphids a true gastrulation obtains, basing his arguments on the fact that the "vitellophags" (my "mycetoblasts") are, as he claims, of entodermal nature. On account of their very early segregation during the time that the cleavage nuclei are apparently still undifferentiated, even prior to the formation of the blastoderm, I cannot subscribe to Will's idea.

Hirschler's (1912) views also seem germane to the present question. He claims that the blastopore (in the sense in which I use the term) is either present or absent in aphids, depending upon the shape of the mass of "pseudovitellus." He says, in part: "In den Fällen wo der Pseudovitellus eine pfropfartige Form besitzt, könnte man die Blastula als am hinteren Eipole offen stehend betrachten, in allen andern, denen alle grossen Blastulae zuzurechnen sind, ist der Dotter allseitig mit Zellen bedeckt, eine Oeffnung ist nicht vorhanden." He attempts to show that Witlaczil's (1884) contention that the blastoderm grows over the entire surface of the egg, and that of Will (1889) and of Tannreuther (1907) that this structure covers only the anterior pole and the sides, leaving a blastopore at the posterior pole, are both correct. In my own work, I have only found the case where the mass of formative "mycetom" serves as a plug ("Pfropf") in the blastopore. I have never had occasion to see the other type.

tities, on account of the fact that the "organisms" have increased so much in number in the egg cavity that a balance must have been almost established by this time. It is hard to account for the persistence of the swollen portion of the follicular epithelium, which continues to increase in size progressively with the succeeding blastodermal stages, even to the time when the formative "mycetom" is so far advanced in development that there could not possibly be any need for reënforcement from that source. One possible explanation is that the stimulus originally exerted by the egg yolk has so activated the "organisms" within the follicular epithelium that rapid multiplication in the latter continues for a long time afterward.

The small anterior nuclei in the stage under consideration in the foregoing paragraphs measure about 3 micra in diameter. They are, of course, also in syncytium, in the cytoplasmic network within the very limited space in the anterior portion of the blastocœle. The "symbionts" apparently do not invade this region.

The formation of the "germ cylinder" through invagination of the blastopore has been described by earlier workers (Will, 1889; Tannreuther, 1907; Hirschler, 1912; et al.). Tannreuther claims to have observed that only the posteriorly located cells, at the rim of the blastopore, give rise to the invaginating cylinder, by proliferation. He has failed to note the fact however that, before invagination takes place, the blastoderm grows posteriorly and fills in the posterolateral gap in the egg chamber, which I have described above. In this way, the lateral sides of the mass of formative "mycetom" are almost completely covered by the blastodermal wall. The posterior extension of the blastoderm apparently results, not only from a multiplication of the cells at the rim of the blastopore, but also from a general division among the constituent cells of the entire blastodermal wall. At the conclusion of extension, the blastoderm (Plate 5, fig. 39, *bl*), in section, appears stratified, as a result of the very considerable increase in the number of cells which crowd together, particularly at the anterior pole. There is also a slight increase in the diameter of the egg.

The mass of formative "mycetom" at this time (Plate 5, fig. 39, *fm*) is irregularly subellipsoidal, completely occupying the posterior half of the total length of the blastocœle, which has become very much greater in diameter at this region. The

formative "mycetom" now adjoins the follicular epithelium posteriorly and is closely applied to the ental surface of the blastoderm laterally. The anterior surface of the mass is sharply defined in outline with the small anterior nuclei, embedded in a dense cytoplasmic mass, contiguous to it. The formative "mycetom" now appears to be much more concentrated, apparently due to the increase in number of "mycetoblasts" present, and their gathering together more closely toward the posterior end of the blastocœle. The "symbionts" are also present in the mass in very much increased proportions, so that the strands composing the cytoplasmic network have become obscured by their presence.[23]

During the subsequent growth of the egg, the "germ cylinder" begins to invaginate, by proliferation of the cells at the rim of the blastopore. Plate 4, figs. 40 and 41, shows two successive stages in the process. In the former figure, there is no evident fundamental change from the previous stage, except for the invaginating blastodermal layer, which becomes closely applied entally to the mass of formative "mycetom." With the subsequent development of the "germ cylinder" (Plate 6, fig. 41, *cyl*) which is open at both ends,[24] the formative "mycetom" is pushed anteriorly as invagination progresses, so that the latter forms a subglobular mass pressed against the anterior end of the

[23] The nuclei in syncytium in a vacuolated layer of cytoplasm which Buchner (1921), using an original drawing by one of his students, represents in his fig. 58b, page 213, as the anlage of the "mycetom," at a stage corresponding to the present one, has not been confirmed in my material. As I have noted above, the entire posterior half of the blastocœle, down to the adjoining follicular epithelium, is occupied by what I have interpreted as a mass of formative "mycetom," the origin of which I have traced from the "mycetoblasts" ("vitellophags") and from the "symbionts" that infect the egg from the follicular epithelium. In the same figure, Buchner has depicted the anterior half of what I have represented in the present paper as the formative "mycetom," as a detached, subglobular mass, which he interprets as the "primitive germ-cells." In their effort to localize the latter cells in this region, Buchner and his student have evidently fallen under the spell of the views of some of the older masters (cf. Henneguy, 1904, p. 405, fig. 395, A, h). The primitive germ cells, at least in the material I worked with, are not in evidence as a definite mass until the "germ-cylinder" is well formed. A fuller description of the first visible appearance of primitive germ cells is given in the text of the present paper.

[24] This condition has been reported first by Will (1889).

former. The swollen follicular epithelium at this stage has developed an anterior cup-shaped depression, the thin edge of which closely fits into the rim of the posterior opening of the cylinder. This structure apparently represents a persistence of the original intimate connection between the egg follicle and the surface of the egg at the posterior pole. After invagination of the cylinder, the pressure of the fluid (amniotic fluid) in its lumen, applied against the anterior wall of the follicular epithelium which is apparently still firmly united with that portion of the blastoderm in contact with it, probably results in this cup-shaped sagging.

The small anterior nuclei (Plate 6, fig. 41, *b*) have, by this time, arranged themselves in a single layer, which is closely in contact with the anterior surface of the mass of formative "mycetom." The former are still distinguishable due to their being relatively smaller than the "mycetoblasts," their more uniformly subglobular shape, the pseudopodlike structure in their surrounding cytoplasm, and also the fact that they are not embedded in the concentrated mass of "symbionts." I have unfortunately failed to trace to my satisfaction the subsequent history of these nuclei beyond this stage, but at no time do they ever show any sign of disintegration. Their persistently close relation to the mass of formative "mycetom" suggests that they may have something to do with the formation of the investing membrane of the definitive "mycetom."

Figure 41 also shows a single layer of cells (nuclei in syncytium?) (*gc*), appearing between the mass of formative "mycetom" and the germ cylinder on one side and the contiguous blastodermal wall on the other. The nuclei are subglobular or subellipsoidal, measuring about 7 to 8 micra in diameter, and possessing distinct nucleoli and less densely staining chromatin granules which, in sections, form a ring almost encircling the former. These nuclei are distinguishable from the "mycetoblasts," near some of which they are located, in that the former have more sharply defined nuclear membranes and, further, like the small anterior nuclei, they do not lie in the mass in which the "symbionts" are concentrated. As may be judged from the figure, there seems to be no question as to their being a distinct layer from the formative "mycetom." Principally on account of their relative position with respect to that part of the "germ cylinder" which in later developmental stages gives rise to the ventral plate of the germ band, I take these cells to be the "primitive germ

cells," which are only becoming distinguishable at this stage.[25] On differentiation of the ventral plate and of the procephalic lobes, which apparently occurs almost simultaneously with the visible differentiation of the mass of primitive germ cells, the last occupies a mesodermal position.

The germ band is formed by a thickening of a certain area at the posterior portion of the blastodermal wall, with a corresponding process in the contiguous part of the "germ cylinder." The rest of the blastoderm and of the "germ cylinder" remain relatively thinner. The thickened posterior part of the blastoderm represents the earlier stages in the differentiation of the procephalic lobes (Plate 6, fig. 41, *pc*) ; the adjoining thickened piece of the "germ cylinder," the ventral plate (*vp*). The amnion (*am*) and the serosa (*se*) are represented by the thinner areas in the "germ cylinder" and in the blastodermal wall, respectively. In the case of the latter, there is also present at the anterior pole a conspicuously thicker layer (Plate 6, figs. 40, 41, *d*), which Will (1889) has called the "Scheitelplatte" and which he has attempted to homologize with the structure known under the same name in the Annelida. He has claimed that this thickened portion eventually migrates posteriorly and joins the ventral plate, thus giving rise to the procephalic lobes. On this basis, he has contended that the anlage of the head has an independent origin from the ventral plate. Wheeler (1893) has justly op-

[25] In the foregoing pages, I have mentioned the fact that certain authors, like Balbiani (Henneguy, 1904, p. 405) and Buchner (1921) claimed to have observed earlier differentiation of the mass of primitive germ cells than I found in the case I have described. I have already noted my objection to Buchner's representation of the "germ-cells" in his fig. 58, *b*, wherein he has apparently confounded the mass of formative "mycetom" with the "germ cells." In his fig. 58, *c* and *d*, he has evidently taken for "germ cells" what I have referred to as small anterior nuclei, which I have traced from an earlier migration from the periplasm before differentiation of the blastoderm. They obviously cannot be "primitive germ cells."

The figure of Balbiani which I have just referred to is too diagrammatic to admit of any adequate discussion in regard to this question.

The position of the primitive germ cells on differentiation, as I have observed it in my material, agrees in a general way with the earlier descriptions of Metschnikow (1866) and of Will (1889). It should be noted that visible differentiation of the mass of primitive germ cells occurs in aphids much later than in coccids. As reported by Pierantoni (1914) and by Shinji (1919), the primitive germ cells in the latter family become distinguishable as fairly large cells at the posterior pole of the egg during early blastodermal stages.

posed this view, particularly in regard to the supposed homology between the aphid brain and the "Scheitelplatte" of annelids, for the former is segmented while the latter is not. This anterior thickening in the aphid blastoderm, so far as I am able to judge from its subsequent history in my own material, has apparently no such important morphological significance as Will has ascribed to it. It seems to be only a reserve mass which allows for a considerable stretching of the serosa as the embryo develops.

In fig. 41, *e* and *f*, both the ventral plate and the amnion are shown with the wall folded over entally at the anterior rim, so that the "germ cylinder" represents at this time a double invagination: (*a*) The primary, or original, anteriorly directed invagination from the rim of the blastopore, resulting in the formation of the main portion of the "germ cylinder;" and (*b*) a secondary, posteriorly directed invagination from the anterior rim of the first. The layer resulting from the latter process is unilaminar, and is closely applied upon the ental wall of the main cylinder. This secondarily invaginating structure is at first open at the posterior rim, but the gap is ultimately sealed over by cell proliferation. In this manner, the "germ cylinder" becomes covered at the anterior opening by a very loose, cellular sheet which is continuous with the walls of the former and which is capable of considerable extension. On completion of this new layer, the mass of formative "mycetom," as well as the rest of the inner cavity of the egg, becomes completely isolated from further direct communication with the follicular epithelium at the posterior pole.

The ventral plate continues to grow anteriorly, its caudal end,[26] in the meantime, pulling with it the adjoining loose cellular sheet which I have described above, as it increases in length. Eventually the latter becomes stretched and, in turn, drags along with it the amniotic part of the "germ cylinder" proper, the two together forming a continuous layer which becomes loosely applied upon the ventral surface of the ventral plate. It is doubtful if the cellular elements of the amnion and of its extension

[26] It is now generally known that the embryo of the aphid, in common with those of other Homoptera, and like those of certain other groups of insects, develops with the head directed at first toward the posterior pole of the egg. Later, however, it orients itself so that the head ultimately points toward the anterior pole (Wheeler's "blastokinesis," 1893).

multiply appreciably; I infer this condition from the manner in which their cells quickly flatten out as a result of stretching. The same statement holds true with reference to the serosa.

As the ventral plate elongates, the mass of formative "mycetom" becomes displaced from its former position. The latter now begins to occupy the large cavity formed by the side of the amnion which is away from the ventral plate, and its contiguous serosa. The "mycetoblasts," which are all apparently in a resting state, in the meantime move farther apart from each other, evidently as a result of expansion of the continually increasing mass of "symbionts."

The primitive germ cells accompany the dorsocaudal portion of the ventral plate, as the latter extends anteriorly. In the meantime, the former tend to collect into a subglobular or abruptly subellipsoidal mass.

I have made no careful observations on the early development of the procephalic lobes. It is evident, however, that they do not increase in size in proportion to the rapid elongation of the ventral plate.

When the egg reaches a length of about 120 micra and a cross-sectional diameter of 60 micra, or approximately twice its dimensions at the time the definitive blastomeres begin to become differentiated, the ventral plate has grown longer than the length of the egg and has curled over posteriorly at the anterior pole of the latter. A lateral view of the germ band at this stage (Plate 7, fig. 45) shows the **S**-shaped appearance, described by Will and by other earlier authors. The differentiation of the ectodermal and the lower layers of the germ band, which must have taken place previous to this stage, is unfortunately very inadequately represented in both my figures (Plate 7, fig. 45, and Plate 8, fig. 48).

The hook-shaped caudal half of the ventral plate occupies the anterior three-sevenths of the total length of the egg. The dorsal surface of the arched portion is closely applied against the serosa at the anterior pole. From the posterior termination of the curvature, the ventral plate is deflected entally at right angles to the longitudinal median axis of the egg, until its caudal end almost touches the contiguous amniotic layer.

A small piece of the amnion which is continuous with the caudal end of the ventral plate is applied anteriorly upon the latter. Distally, the former is connected with a still shorter

portion of the amnion, which is deflected anteriorly at an angle of about 30° to 45° and is in turn joined by the main amniotic membrane. The latter piece runs anteroposteriorly between the anterior porrect half of the ventral plate on one side, and the latter's deflected caudal portion, the mass of primitive germ cells, and the formative "mycetom" on the other.

The primitive germ cells at this time form a subglobular mass (Plate 7, fig. 45; Plate 8, fig. 48, *gc;* Plate 3, fig. 19) which fits into the concavity in the deflected caudal piece of the ventral plate. The nuclei of the former are subglobular, measuring about 5 micra in diameter and each, with few exceptions, containing sparsely scattered, darkly staining chromatin granules and a distinct nucleolus. Some of the cells are apparently in early prophase.

Adjoining the foregoing region posteriorly is the formative "mycetom," which now occupies about one-third the total volume of the egg. This mass is bounded ectally and posteriorly by the serosa and entally by the amnion. The "mycetoblasts" measure about 6 micra in diameter and are characterized, as before, by the presence of sparsely distributed chromatin granules and of large and distinct nucleoli. This stage of embryonic development appears to cover the period in which the differentiation of the definitive cell territories of the "mycetom" takes place. The process apparently begins by the "mycetoblasts" first exhibiting signs of renewed mitotic activity (Plate 8, fig. 47), after which the mass of "symbionts" and the cytoplasmic network become constricted between the nuclei, thus marking out the ultimate cells.

Figure 45 also shows the persisting swollen portion of the egg follicle.

One other important feature in connection with this stage, as represented by figs. 45 and 48, is the fact that the invagination of the stomodæum takes place at about this time. The proctodæum does not begin to invaginate until later.

SUMMARY AND CONCLUSIONS

1. The periplasmic layer apparently foreshadows the future position and the extent of formation of the blastodermal wall.

2. The definitive cell territories of the blastoderm are formed by simple, simultaneous construction of the periplasm, between the nuclei which are embedded in the latter. A secondary "Keimhautblastem," which has been described in other groups of insects, has not been found in aphids.

3. Differentiation of the definitive cell territories in the blastoderm is accompanied by no appreciable increase in the size of the egg.

4. At first, the blastodermal wall occupies only the anterior and a fraction of the lateral sides in the periphery of the egg, leaving a small portion toward the posterior pole uncovered. By subsequent growth, however, the blastoderm eventually extends posteriorly until the entire length of the lateral sides of the egg is covered.

5. The blastoderm leaves an opening, or blastopore, at the posterior pole of the egg, at the region of the original gap left by the periplasm during the preceding cleavage stages.

6. Invagination of the "germ cylinder" is effected by proliferation of the cells at the blastopore. The "germ cylinder" at first is open, both anteriorly and posteriorly.

7. A longitudinal thickening of a part of the wall of the "germ cylinder" gives rise to the ventral plate of the germ band; a corresponding thickening of a contiguous portion in the blastodermal wall, to the procephalic lobes.

8. The thinner part of the "germ cylinder" represents the amnion, and a contiguous thinner portion in the blastodermal wall, the serosa. There is a thickening of the blastodermal wall, at the anterior pole, opposite the blastopore, but this apparently has no further morphological significance than an accumulation of reserve mass in the serosa, which allows for considerable expansion of that embryonic envelope during the subsequent developmental stages.

9. A secondary, posteriorly directed invagination occurs at the anterior rim of the "germ cylinder," resulting in the formation at this region of a loose cellular sheet covering the opening. This structure is capable of considerable extension, and ultimately becomes a continuation of the amniotic part of the "germ cylinder," thus adding very materially to the total expansive area of this particular embryonic envelope.

10. The ventral plate continues to elongate anteriorly until ultimately it exceeds the total length of the egg. Then its caudal half curls over posteriorly at the anterior pole, the ventral plate halfway doubling over itself around its ventral surface in this manner.

11. The procephalic lobes do not elongate in proportion to the growth of the ventral plate.

12. During these earlier stages, the cephalic region of the embryo is directed toward the posterior pole of the egg. Later,

however, the embryo turns around so that the head finally points toward the anterior pole, through a process which has been variously called blastokinesis, rotation, or orientation.

13. The "symbionts" which invade the egg cavity apparently become lodged within the meshes of the cytoplasmic network where they continue to multiply and, in the meantime, gradually appropriate the yolk.

14. After differentiation of the blastoderm, the inflow of the "symbionts" into the egg probably begins to diminish, as a result of the fact that a balance in concentration between the "symbiotic" mass in the egg cavity and that in the swollen portion of the follicular epithelium at the posterior pole is rapidly being approached.

15. The "mycetoblasts," which are in syncytium in the cytoplasmic network, together with the "symbionts," collect into a large, compact, subglobular mass at the posterior half of the blastocœle. The mass constitutes the formative "mycetom."

16. The formative "mycetom" is progressively pushed anteriorly in the blastocœle by the invaginating " germ cylinder," close to the anterior opening of which this organ is located.

17. As the ventral plate elongates, the loose cellular sheet at the distal end of the "germ cylinder," together with the amniotic part of the latter, is stretched anteriorly. As a result of the process, the formative "mycetom" becomes displaced in the cavity formed by the amnion and its contiguous serosa, at the posteroventral side of the germ band. While in this cavity, the "mycetoblasts" become widely dispersed, apparently owing to a rapid increase in mass of the "symbionts."

18. Definitive differentiation of the cellular elements of the "mycetom" occurs after the time when the ventral plate has curled over at the anterior pole of the egg, as summarized in paragraph 10 of this summary. Prior to cellular differentiation, the "mycetoblasts" exhibit renewed signs of mitotic activity.

19. The swollen portion of the follicular epithelium persists to this stage, when obviously there is no further need of reenforcement in the supply of "symbionts" from this source. This persistence is probably the result of a prolonged after effect of the original stimulus, exerted by the egg yolk during the earlier stages in the development of the egg.

20. The small anterior nuclei, which have originated from a secondary migration from the periplasm into the anterior portion of the egg cavity during the time that the definitive blastomeres are being formed, show no signs of degeneration during

any of the stages in which they are recognizable. Unfortunately, their actual ultimate disposal in the later embryonic stages has not been followed. Their very close association with the mass of formative "mycetom" suggests that they may have something to do with the formation of the investing membrane of that organ.

21. The mass of primitive germ cells is not in evidence until late in the blastodermal stages, almost simultaneously with the differentiation of the procephalic lobes and of the ventral plate. The mass of primitive germ cells at first appears in the form of a single, unilaminar layer, which is mesodermal in position, and which always follows the dorsocaudal portion of the ventral plate as the latter proceeds in its elongation. At a later stage, the primitive germ cells gather into a subglobular or abruptly subellipsoidal mass in the same relative location with respect to the ventral plate.

THE LATER EMBRYONIC STAGES

In the present account of the later stages in the development of the embryo, only the "mycetom" is taken up in detail. The other principal organs are merely mentioned in passing or characterized in general terms, with fragmentary notes used as supplement. A more thorough treatment of the various other parts of the embryo has of necessity been deferred, on account of the present incomplete state of my data relating to them.

Metameric segmentation first becomes manifest in the appearance in the germ band of wavy swellings, which extend to both the procephalic lobes and the ventral plate (Plate 7, fig. 44; Plate 8, fig. 49). In general, the process is similar to the condition which Graber (1888, 1890) has described in Diptera. Figure 49, of the present paper, which represents a sagittal section, sinistral aspect, of an egg of an unidentified species on *Lythrum*, shows a relatively more advanced stage, wherein the anlagen of appendages begin to appear. The "mycetom" remains in the same position that it occupied prior to metameric segmentation—in the cavity which lies posteroventrally with respect to the germ band, and which immediately adjoins the flexed caudal portion of the ventral plate.

The series represented by figs. 45, 48, 44, and 49 unfortunately has not been drawn from a single species. The figures show, in the order given here, the successive steps in the movement of the caudal end of the ventral plate. After assuming the curled-up position shown in fig. 45, the caudal piece proceeds to grow

posteriorly, replacing the amnion by degrees, until the former finally occupies the original position of the latter close to the ental surface of the "mycetom." The anteroposteriorly descending portion of the ventral plate represents the abdominal region of the embryo. Its caudal end now lies only a little anteriorly to, and opposite the opening of, the stomodæum. Plate 9, fig. 51, and Plate 10, figs. 52 and 53, show the embryo after the abdominal region has assumed this position. Plate 1, figs. 3 and 4, represents corresponding surface views of embryos of about the same stage. The rudiments of the mouth parts and legs develop as ectodermal evaginations (Plate 9, fig. 51; Plate 10, figs. 52 and 53, *fe;* and fig. 54). The cavities thus formed are filled with mesodermal tissue (*ms*), which gives rise to muscles.

The mass of germ cells, which is constantly associated with the dorsocaudal end of the ventral plate, apparently begins to divide at this time into separate lots, each to be ultimately inclosed in a definitive germarium. The details of this partition have never been followed satisfactorily in aphids, and our knowledge in regard to the differentiation of the definitive ovarian parts in this group of insects still leaves much to be worked out. In certain favorable sections, I have noted a thick mesodermal layer (Plate 9, fig. 51, *ms*) covering portions of the surface of the mass of germ cells. By analogy with the Coccidæ, in which family the development of the various parts of the ovaries has been more satisfactorily studied (Pierantoni, 1914), this mesodermal layer probably gives rise to the parts of the ovary distally adjoining the vagina. According to Pierantoni's account, that mass of cells which I have interpreted in the present work as formative egg follicle also arises from the mesoderm, and it is not improbable that a similar process also occurs in so closely related a group as the aphids. The intimate association of the mesodermal layer referred to above with the "symbiotic organ" suggests the possibility of early infection during this time of the developing ovaries by the "symbionts."

The oögonia apparently arise directly from the primitive germ cells and are inclosed as such, by the definitive germarium, in much larger numbers than are needed for the production of ovarian eggs.

The proctodæum (Plate 9, fig. 51; Plate 10, figs. 52, 53, *pr*) apparently invaginates at this stage. The blind end of this invagination, as well as that of the stomodæum, which arises earlier, is adjoined by a thick layer of mesentoderm. The last

presumably gives rise to the epithelial lining of the mesenteron, as described by Hirschler (1912).

The differentiation of the brain (*br*) from the procephalic lobes and of the subœsophageal and thoracic ganglia and ventral nerve chain from the ventral plate also becomes apparent at this time. In slightly older embryos (fig. 53, *ad*), adipose tissue is also in evidence along the dorsal part of the thoracic region.

A subglobular mass of cells (Plate 9, fig. 51; Plate 10, figs. 52, 53, *g*), which appears to be composed of a thick outer wall, inclosing an irregular cellular layer, appears at the opening of the stomodæum. Its position, as well as its shape, is very suggestive of the indusium, which Wheeler (1893) has described in *Xiphidium* and which has also been reported subsequently in certain Homoptera, as in *Siphanta* (Muir and Kershaw, 1912). In as much as, in the aphids, I have been able to trace neither the origin nor the subsequent history of this peculiar structure, I shall make no attempt definitely to establish its homology until I have had a chance to give it more thorough study.

The "mycetom," as in the preceding stage, occupies approximately one-third the total volume of the egg chamber, but it is now distinctly anterior in its position (Plate 10, figs. 52, 53, *my*). The developing embryo tends progressively to shift directly underneath this organ. The cellular elements of the "mycetom" ("mycetocytes") are clearly distinguishable. They number at this stage from about thirty to forty, and are yellowish green in fresh material, on account of the coloring matter of the "symbionts." The "mycetocytes" measure from 18 to 24 micra in diameter. The nuclei are subglobular, with distinct nucleoli, and a few dark-staining, widely scattered chromatin granules.

The last stage described above appears to be the one which immediately precedes orientation of the embryo. The egg of *Macrosiphum tanaceti* reaches a length of about 230 to 280 micra and a cross-sectional diameter of about 130 to 165 micra before the process takes place. My deductions from study of the aphid material I have on hand confirm in essential respects Brandt's (1869) description of orientation in this family. Pierantoni (1914) has also described a similar process in *Pseudococcus citri* Risso (Coccidæ). It takes place as follows: As the caudal end of the abdomen descends posteriorly to the position described above, in front of the stomodæum, the amnion becomes gradu-

ally released from its intimate connection with the "mycetom." The serosa is contiguous with the amnion. At the point of junction with the latter, an extension of the fold overarches the posterior opening of the "germ cylinder," so that the serosa appears to cover the entire periphery of the egg, except the procephalic lobes. As the amnion becomes detached from the ental surface of the "mycetom," the serosa contracts and draws the former with it, so that the two embryonic envelopes form a continuous, ectal amnioserosal membrane. Contraction proceeds, and the resulting anteriorly directed force causes an eversion of the embryo from its original, ventrally flexed posture. A surface view of an embryo of *Myzus persicæ* Sulzer at the conclusion of blastokinesis is shown in Plate 1, fig. 5. The mouth parts and the legs are still relatively short and undeveloped. The characteristic position of the abdomen, which is dorsally curved, as a result of rotation, is shown. Plate 11, fig. 56, represents a somewhat more advanced stage in *Macrosiphum tanaceti,* after the dorsal abdominal wall has been formed, replacing the amnioserosal membrane, which has disintegrated. It will be noted that, as a result of rotation of the embryo, the "mycetom" has moved from its anterior position, as shown in Plate 10, fig. 53, and settled into the cavity formed by the dorsal curvature of the abdomen. Up to this time, this organ persists as a single mass of cells, which apparently exert very little mutual pressure, a condition which I infer from their relatively more subglobular form. The twelve newly formed ovarioles, five of the germaria of which are shown in fig. 56, are arranged bilaterally in two groups, each embedded in either posterolateral side of the "mycetom." A median, sagittal section of the abdomen at this stage, showing the posterodorsally located opening of the proctodæum, is represented in Plate 12, fig. 57. The proctodæum in the figure includes only a small portion adjoining the opening.

The union of the stomodæum and the proctodæum into one single, continuous digestive tube takes place soon after orientation. I have not studied the details of the process. Hirschler (1912), however, after making a searching examination of the work of previous authors and correlating them with his own results, observes that the "Stomodäum und Proctodäum . . . . grösstenteils den Darmtractus aufbauen," while the entoderm (mesentoderm), a layer of which has been pointed out before as adjoining the blind ends of both the stomodæum and the proctodæum, "in den kleinen, unansehnlichen Mitteldarmanlagen re-

präsentiert, deren prospektive Bedeutung bei weitem geringer ist und die sich nur am Aufbaue der Darmschlinge beteiligen." Similar conclusions have been reached by Pierantoni (1914) in regard to the limited part played by the mesentoderm in the formation of the alimentary canal. Hirschler, therefore, contends that the anterior bulb-shaped enlargement of the digestive tube, which has often been taken for the mid-intestine (Plate 1, fig. 11; Plate 2, figs. 13, 14; Plate 7, fig. 46; Plate 13, fig. 61 *mi*), is really a modification of the foregut.

With the further development of the alimentary canal, the pressure which the foregut exerts posterodorsally and the hindgut anteroventrally on the mass of "mycetom" is apparently responsible for the bipartition of the latter into two longitudinal halves. A few dorsally located "mycetocytes" connect the two lobes of the "mycetom" subcaudally. This bridge, however, disappears later, during the nymphal stage, as the pressure exerted by the growing alimentary canal increases in that region. Plate 11, fig. 55, and Plate 13, fig. 62,[27] show two successive steps in the division of the "mycetom." Figure 55 is a cross section of the abdomen, passing through the bulb-shaped enlargement of the foregut. The embryo is practically of the same age as the one shown in fig. 56. The bipartition of the "mycetom" is only beginning to take place. Figure 62 is a section through the subcaudal portion of an older embryo, which is at about the stage shown in Plate 1, figs. 6, 7, and 8. The separation of the two lobes of the "mycetom" is nearly complete.

Figure 62 is one of the series of cross sections of an embryo which, in cephalocaudal order, are arranged as follows: Figs. 58, 59, 60, 61, and 62. The first two are sections of the cephalic region, showing the development of the various parts of the brain, and also of the mouth parts, including the "retort organs," at this stage. The "retort organs," which have been known for a long time (Metschnikow, 1866; Witlaczil, 1882; Zacharias, 1884, et al.), are present in two pairs, one in each side of the head. Plate 1, fig. 9, *ro*, also shows this structure.

Figures 60 and 61 are sections through the thoracic region. The first passes through the salivary glands and the subœsophageal ganglion; the second, through a portion of the bulb-shaped anterior enlargement of the foregut and one of the thoracic ganglia. The "mycetom" does not extend into the thoracic cavity.

[27] The former represents *Macrosiphum tanaceti;* the latter, *M. rosæ.*

The arrangement of the "mycetom" in fig. 62 has already been described. A sagittal section of an embryo of the same stage, showing the dorsally located layer of "mycetocytes," is represented in Plate 7, fig. 46. A frontal section, through the nervous system, is shown in fig. 63. The cerebral lobes and other parts of the brain, and the subœsophageal and thoracic ganglia are very well developed at this stage. The abdominal cavity lateral to the nervous system is occupied mainly by adipose tissue; no "mycetom" is evident in this region.

A full-grown embryo of *Macrosiphum tanaceti,* which is about ready to be born, is shown in Plate 1, fig. 10, in surface view, and in fig. 11, a sagittal section a little to one side of the median line of the body. The embryo now measures about 840 micra in length; 264 micra in width; 240 micra in thickness, exclusive of legs; and 300 micra, with legs in natural position. The legs have grown considerably in length, so that the metathoracic pair curves around the caudal third of the abdomen to the other side. The haustellum has assumed its definitive tubular structure. The ungues and body hair are fully in evidence, and a general chitinization of the body wall is apparent in this, as well as in an earlier stage represented by fig. 9, so that they no longer readily take the stains which the younger embryos show beautifully.

The nerve chain, composed of the brain (*br*), the subœsophageal ganglion, and the thoracic and abdominal ganglia (Plate 1, fig. 11, and Plate 2, fig. 12, *ta*), is much longer in proportion to the body, as compared with that of the later nymphal and of the adult stages. The œsophagus (fig. 11, *oes*) extends from the pharynx dorsally and, from this vertical position, it bends posteriorly and joins the enlarged bulb-shaped portion of the digestive tube (*mi*). The point of junction of the œsophagus with this bulb-shaped enlargement is guarded by a posteriorly directed valve (Plate 2, figs. 13, 14, *vlv*). This enlargement, according to Hirschler, is not the mid-intestine, but is simply a modification of the foregut. The posteriorly adjoining part of the digestive tube is coiled into a single loop, and it is in a small portion of the latter that the mesenteron is represented.

The "mycetom" (fig. 11, *my*) is completely divided into two longitudinal halves, except at the subcaudal end, as before. The "mycetocytes" number from about sixty to seventy, indicating thus that the cells must have divided at least once since the first formation of the definitive cell territories of the "mycetom." They are subglobular or subpolygonal, as a result of mutual

pressure, and measure about 45 micra in diameter. The "symbionts" are congested in the cytoplasmic region and, as in the preceding instars, obscure the cytoplasmic network, on account of their presence in enormous numbers. The nuclei measure about 10 micra, and present an irregularly subellipsoidal or subpolygonal aspect, with a densely staining, granular, chromatin network, and a large, distinct, subglobular nucleolus. The thin investing membrane is distinguishable here and there, on account of its narrowly subellipsoidal nuclei, among the "mycetocytes;" but I have been unable to determine whether there is a separate enveloping layer covering each "symbiotic" cell, or whether several cells are inclosed together by a common membrane. The investing membrane is apparently not continuous around the entire "mycetom."

One other interesting point in connection with the full-grown embryo is the fact that its enveloping follicular epithelium shows no signs of degeneration (Plate 1, fig. 11, *fe*). Plate 2, fig. 17, shows a portion of these epithelial cells (*fe*) which, although very widely stretched, apparently still retain perfect nuclear organization. The nuclei are elongately subellipsoidal, about 5 micra long, and filled with numerous, darkly staining chromatin granules. The cells are spindle-shaped, and measure about 70 micra in length and 1.5 micra in thickness. The persistence of the egg follicle to this late stage suggests that this tissue is probably the membranous sheet which envelopes the nymph at birth.

SUMMARY AND CONCLUSIONS

1. Metameric segmentation of the germ band begins to become manifest in the form of wavy swellings in both the ventral plate and the procephalic lobes.

2. After assuming the curled-up position at the anterior pole of the egg, the flexed portion of the ventral plate proceeds to grow posteriorly, and ultimately replaces the amnion at the ental wall of the "mycetom." The posteriorly flexed portion of the ventral plate at this time represents the abdominal region of the embryo.

3. As the amnion becomes dislodged, the serosa contracts, drawing the former ectally, so that the two embryonic envelopes form a continuous amnioserosal sheet. A progressive contraction of the combined envelopes apparently results in the rotation of the embryo, through anteriorly directed pull, causing eversion of the latter from its original, ventrally flexed position.

4. The rudiments of the appendages and of the internal organs are fully differentiated before orientation takes place.

5. The invagination of the proctodæum also occurs prior to blastokinesis. As stated previously, the stomodæum invaginates earlier, before metameric segmentation of the germ band.

6. The union, into one continuous digestive tube, of the foregut and the hind gut, by means of the mesenteric epithelium, which arises from the mesentoderm accompanying the blind ends of both the stomodæal and the proctodæal invaginations, occurs some time after blastokinesis.

7. Prior to orientation, there appears at the opening of the stomodæum a subglobular, cellular structure which is very suggestive of Wheeler's indusium. Its homology with the latter, however, cannot be established definitely at present, on account of the doubtful origin and subsequent history of this peculiar formation in aphids.

8. The persistently close association with the "mycetom" of the mesodermal layer which presumably gives rise to the various parts of the ovary distal to the vagina, apparently including the formative egg follicle, tends to bring about a condition wherein early infection of the last tissue by the "symbionts" may take place. In fact, on account of the constant intimate contact of the germ band with the formative "mycetom" as a result of which the lower layer of the ventral plate, from which the mesoderm in question develops, is bathed by the fluid loaded with "microörganisms," there is reason to believe that infection probably begins at an even earlier stage.

9. The oögonia apparently arise from the primitive germ cells, and are inclosed as such in the definitive germaria. They do not, as certain authors claim, originate from the egg follicle.

10. The apportionment of the oögonia into lots, each occupying one of the twelve germaria, apparently occurs prior to blastokinesis.

11. After orientation of the embryo, the ovarioles are embedded among the superficial cells of the "mycetom" at each lateral side.

12. As a result of the posteriorly directed growth of the flexed portion of the ventral plate, the "mycetom" is pushed from its original position at the anteroventral side of the embryo, near the posterior pole, to the anterior third of the egg. Accompanying blastokinesis, the "mycetom" is dislodged again from the latter position and shoved posteriorly into the dorsal curvature of the abdomen of the embryo.

13. The "mycetom," at first a single mass of "mycetocytes," becomes eventually divided into two lateral halves, apparently as a result of pressure exerted by the developing digestive tube, after orientation.

14. The "mycetocytes" appear to undergo cell division at least once during the embryonic stages, subsequent to definitive differentiation from the formative "mycetom." The resulting increase in number is from about thirty to forty to about sixty to seventy. The cells have grown in size also, from about 18 to 24 micra, immediately following differentiation, to about 45 micra in the oldest embryo.

15. The egg follicle apparently does not degenerate, but persists throughout the last embryonic stages. Such a condition indicates that this tissue is probably the origin of the thin, membranous envelope covering the nymph at birth.

THE "MYCETOM:" MORPHOLOGY AND DEVELOPMENTAL HISTORY IN THE POSTNATAL STAGES OF APHIDS

The "mycetom" of the newly born nymph, like that of the later prenatal stages, is a large mass of longitudinally bipartite organ, which extends from about the second to about the sixth or seventh abdominal segment. Each lobe measures, in lateral diameter, roughly one-fifth the greatest width of the abdomen, and occupies the cavity on either side of the alimentary canal. The organ lies nearer the dorsal than the ventral abdominal walls. The cellular investing membrane, which I have described before in connection with earlier stages, is still in evidence; this structure, also present in the "mycetom" of the adults (Plate 9, fig. 50, *im*), apparently persists during the entire life of the aphid.

As has been described in older embryos, a few "mycetocytes" connect the two lobes of the "mycetom" of the young nymph subcaudally above the rectum, and the organ at this time thus presents from the dorsal aspect a horseshoe-shaped form. This condition continues through about the second instar in *Macrosiphum tanaceti*. During the third instar, the connecting bridge disappears, presumably through increased pressure accompanying the further increase in diameter of the digestive tube in this region.

The developing ovarioles, as before, are embedded in the ectolateral sides of the lobes of the "mycetom," among the more superficial "mycetocytes." With the development of the eggs, which at the same time continually increase in number by

additional extrusion from the germaria, the abdominal cavity soon becomes overcrowded so that the "mycetom" is compressed. The result is that, at about the fourth instar, the latter organ is reduced to isolated groups of two, three, or more cells, lodged in the interstices formed by adjacent ovarioles. Later, many of the "mycetocytes" are forced out, partly to the anterior and partly to the subcaudal region of the abdominal cavity, contiguous to the anterior and the posterior ends of the groups of ovarioles, respectively. Ectally, the "symbiotic" as well as the other internal organs in the abdomen are bounded by a thick layer of adipose tissue, which lines the inner wall of the body cavity.

With the exception of certain differences which I am pointing out in the footnote, I have confirmed some of the important details in Witlaczil's (1882) earlier description of the "mycetom." This author bases his account on "*Aphis pelargonii, A.* (*Drepanosiphum*) *platanoides, A. sambuci, Chaitophorus populi, Pemphigus bursarius,*" and several other species of aphids. He reports that this organ—

> liegt seitlich im Abdomen in Form zweier Stränge, die zwischen den dorso-ventral verlaufenden respiratorischen Muskeln der Abdominalsegmente sich hinziehen. Im ersten Abdominalsegmente beginnend, gehen diese Stränge zwischen den erwähnten Muskeln sich immer verengernd durch das zweite, dritte, vierte und fünfte Segment, und vereinigen sich oberhalb des Enddarmes ungefähr im sechsten Segmente, in eine Spitze nach hinten auslaufend, die * * * mit dem Enddarme zusammenhängt.[28]

Witlaczil has also reported the peculiar arrangement in certain species, like *Callipterus tiliæ* Linnæus, in which the two lobes of the "mycetom" are joined by the connecting bridge anteriorly instead of subcaudally. I have never come across this interesting condition in any of my material.

There is no evident direct connection between the "mycetom" and the contiguous organs, in the form of tubes, ducts, or other similar accessories, except the tracheoles of the respiratory

[28] As will be noted by comparing Witlaczil's account with mine, I disagree with him in that, according to my observations on *Macrosiphum tanaceti*, the "mycetom" extends from the second to the sixth or seventh abdominal segment, instead of from the first to the sixth. Further, it is evident that Witlaczil has based his description entirely on the younger nymphs, as may be judged from his failure to notice the disappearance of the subcaudal connecting bridge of the two lobes of the "mycetom" at about the second instar. There is, likewise, no mention in his paper of the ultimate dispersal of the organ into isolated groups of cells, as a result of compression by the developing eggs.

system. The tracheation of the "mycetom" can be studied in favorable sections. As shown in Plate 9, fig. 50, the points where the tracheoles join this organ are apparently without process, the termination of the former being characterized by a simple opening. These tracheoles are relatively large, measuring about 7 micra in cross-sectional diameter. The size is probably correlated with very active metabolic processes which go on within the "mycetocytes," acompanying the presence of the "symbiotic organisms." Large conducting tubes are, therefore, presumably required for securing an adequate supply of oxygen for this organ and, as Pierantoni (1910a) has previously suggested, for elimination of waste gaseous products which result from the chemical action brought about by the "symbionts." I have not been able to determine whether each individual "mycetocyte" is supplied with a tracheole, or two or more of these cells have a common source of air supply. Of the total of nine pairs of spiracles [29] present in *Macrosiphum*, only seven abdominal pairs apparently have direct connection with the respiratory equipment of the "mycetom."

[29] Witlaczil (1882) contends that in the species he worked with the first pair really belongs to the mesothorax, and that the prothorax is without spiracles. He says: "Das erste Stigma liegt seitlich auf der Grenze von Pro- und Mesothorax und ist wahrscheinlich * * * nur nach Verlust des Stigma des Prothorax nach vorn gerückt, gehört aber dem Mesothorax an. Die folgenden Stigmen liegen auch seitlich von Vorder- und Hinterrand des betreffenden Segments ziemlich gleich weit entfernt und zwar das zweite im Metathorax und die folgenden in den ersten sieben Abdominalsegmenten. Die drei letzten Segmente sind ohne Stigmen."

My own findings on *Macrosiphum tanaceti* appear to agree with Witlaczil's. In *Macrosiphum* the spiracles are arranged parallel to the lateral margins of the dorsal sclerites and, as Bonnet (1779) has pointed out previously in other species of aphids, are in line with the bases of the cornicles. The area which borders the rim of each stigmatal opening is piceous, concolorous with the legs, and the location is marked by an abrupt depression in the body wall, so that its presence can hardly escape observation. The spiracle (Plate 2, fig. 15) is characterized by the elevation and partial arching of one-half of the tube over the rim of the other half. The latter portion is marked by a contiguous, superficial groove, producing the rim into a moderately thick, ectally directed ridge, which is subconfluent entally with the adjoining wall of the trachea and almost touches the wall of the other half. The groove and the ental portion of the opposite wall bear long hairs, which are matted together and which presumably protect the tracheal opening by keeping off dust particles. The average diameter of the stigmata in adult *Macrosiphum* is 2 micra.

The component cells of the "mycetom" in the newly born nymph are subglobular, as in the latest prenatal stages and, like those of the latter, show no evidence of very much mutual pressure. They measure from about 42 to 45 micra in diameter, with an average of 44 micra, or almost equal to the measurements of these cells shortly before birth of the aphid. The nucleus is irregularly subglobular, and measures from 9 to 12 micra in diameter. The nucleolus is large and stains deeply in Ehrlich's acid hæmatoxylin. The chromatin network is densely arranged, giving the karyolymph a dark, granular appearance. The nuclear region is never invaded by the "symbionts." As in the later embryonic stages, the "symbionts" have so crowded together in the cytoplasmic mass that the latter has been reduced to an extremely delicate, almost imperceptible, interwoven strand, sustaining the "organisms." On casual examination, one would gain the impression that the "symbionts" have so completely occupied the space between the nuclear membrane and the periphery of the "mycetocyte" that even the intervening cytoplasmic strands have disappeared.

In the adult, the apparently normal "mycetocytes" (Plate 6, fig. 43) measure from about 72 to 108 micra, with an average of about 92 micra. These figures represent an increase of about two or two and one-half times the size of "mycetocytes" of the newly born nymphs and of the older embryos. The persistent closely packed condition of the "symbionts," even in the "mycetocytes" of the adult, indicate that the "organisms" have multiplied correspondingly. The increase in size of the "mycetocyte," however, is not due to increase in number of these cytoplasmic inclusions alone, for the cell itself must have grown also. I infer the latter condition from the fact that the nucleus of the "mycetocyte" has correspondingly increased in diameter from 9 to 12 micra in the later embryonic and earlier nymphal stages, to about 18 to 20 micra in the adult.

Except in size the nuclei of the adult "mycetocytes" are identical in structural characters to those of earlier instars. As the aphid reaches the adult stage, some of those nuclei become so irregular as to appear almost amœboid, while others assume a narrowly elongate form. This condition is probably brought about by increasing mutual pressure of the "mycetocytes" as they grow in size, or to their being more tightly compressed by the developing embryos.

My criterion in judging normal "mycetocytes" is principally the green color of the cells, which is due to granular, probably

chlorophylloid, inclusions of the "symbionts." These green cells are more numerous than the others, which are described below, and the "microörganisms" within them are more compactly arranged.

The other kind of "mycetocyte" measures from about 131 to 162 micra in diameter. It is yellowish or greenish brown, and readily breaks enabling the contents to ooze out rapidly when the fresh aphid is dissected in Ringer's solution. These cells are less numerous than the green "mycetocytes" described above and they are interspersed among the latter. I interpret these brown "mycetocytes" as cells which degenerate after having completed their term of service. The yellowish brown color is apparently due to the loss of green coloring matter by the "symbionts," presumably as a result of untoward conditions in the cells causing death of the former.

I am inclined to accept Flögel's (1905) view that the "mycetocytes" do not undergo further cell division after birth of the nymph, but that they merely increase in size. Neither in the adults nor in any of the nymphal instars have I found indications either of karyokinesis or of amitosis, although I have looked particularly for them. Contrary to the present observations are the findings of Šulc (1910), who claims to have noticed mitosis among the "mycetocytes" of adult *Aphis amenticola* (?) Kaltenbach. He reports that "zur Zeit, wo die meisten Eier reifen oder sich im Leibe des Tieres in Furchung befinden (vivipare Arten) auch im 'Pseudovitellus' (meine Mycetocyte) reiche Furchungsfiguren zu finden sind." I have not been so fortunate in any of the species I worked with, although in some of the adults I have noted that many of the nuclei of the "mycetocytes" are very much distorted, so that in some cases their form resembles superficially certain mitotic phases, particularly the late anaphase and the telophase.

The "mycetocytes" present in an individual aphid number about sixty to seventy in the nymphal instars, as in the older embryos. Soon after reaching the adult stage, the cells begin to degenerate, one by one until, finally, the mother, exhausted as a result of her rôle of producer and about to die, has only a few "mycetocytes" remaining within her body.

The "mycetom" is also present in the amphigonous forms, both in the male and in the female.

SUMMARY AND CONCLUSIONS

1. The "mycetom" of the newly born nymph is a longitudinally bipartite organ, extending from about the second to about

the sixth or seventh abdominal segment. Each lobe occupies the cavity on either side of the alimentary canal.

2. The bridge of "mycetocytes" which connects the subcaudal portion of the two lobes of the "mycetom" persists through about the second instar. It disappears during the third instar, presumably as a result of increased pressure exerted by the growing digestive tube at this region.

3. As the aphid passes to the fourth instar, the "mycetom" is reduced to isolated groups of two, three, or more "mycetocytes," located anteriorly and posteriorly with respect to the ovarioles and in the interstices formed by the adjacent portions of the latter. This condition apparently results from compression accompanying the rapid increase in number and continued development of the eggs, which become progressively overcrowded in the abdominal cavity.

4. The investing membrane of the "mycetom" persists throughout the life of the aphid.

5. The "mycetom" is supplied with relatively large tracheoles, measuring about 7 micra in diameter. The increased metabolic activity accompanying the presence of the "symbionts" in this organ presumably requires these large-sized conducting tubes, in order to insure a plentiful supply of oxygen, as well as to provide for elimination of poisonous gases.

6. There is no increase in the size of "mycetocytes" accompanying birth of the aphid. The "mycetocytes" of the newly born nymphs measure from about 42 to 45 micra, with an average of about 44 micra.

7. After birth of the aphid, the "mycetocytes" apparently do not undergo further cell division. They increase in size only, due partly to increase in the number of the "symbiotic" cytoplasmic inclusions, and partly to growth of the cell itself.

8. The total number of "symbiotic" cells present in each individual aphid during the nymphal instars ranges, within fairly uniform limits, from about sixty to about seventy, as in full-grown embryos.

9. By the time the adult stage is reached the cells measure from about 72 to about 108 micra, the average being 92 micra. These figures represent an increase of about two to two and one-half times the dimensions of the "mycetocytes" of the newly born nymphs and of the older embryos.

10. After reaching the adult stage, the "mycetocytes" degenerate, one by one, until toward the close of the life of the

aphid only very few of these cells are left in her abdominal cavity.

11. The "mycetom" is present also in the amphigonous forms, both in the male and in the female.

GENERAL SUMMARY

The follicular epithelium, which apparently becomes infected by the "symbionts" during the early stages at about the time of or before differentiation of its formative cells, harbors these "microörganisms," evidently in a dormant condition. On contact with the egg yolk, during the earlier cleavage stages of the egg, the dormant "symbionts" become suddenly stimulated. The stimulus brings about the rapid multiplication of the latter, which causes considerable swelling in the portion of the follicular epithelium adjoining the posterior pole. As a result of this activity, the "symbionts" break through the thin epithelial cells and invade the posterior portion of the egg cavity through the posterior opening in the periplasm. They occupy the meshes of the cytoplasmic network and appropriate the yolk mass which is inclosed there.

The "vitellophags," which I have called "mycetoblasts" in the present work on account of their subsequent, apparently more important rôle, show marked attraction to the "symbionts" and tend to aggregate in the posterior half of the egg cavity. The attraction of the "mycetoblasts" to the inflowing granular mass, which process is very suggestive of the behavior of leucocytes in the presence of foreign protein (phagocytosis) as well as the stimulation of these granules in the follicular epithelium on contact with the egg yolk, tends to add weight to the contention that the supposed "symbionts" are really extraneous microörganisms, and not by-products of the insect's own metabolism.

The "mycetoblasts" form a subglobular mass, together with the cytoplasmic network, in which they are in syncytium, and the inclosed "symbionts." In this manner the anlage of the "mycetom" is established. Definitive cellular differentiation of the formative "mycetom" takes place after an active mitotic division of the "mycetoblasts," shortly before metameric segmentation of the germ band. At first existing as a single mass of "mycetocytes," this organ becomes divided into two lateral halves, apparently as a result of pressure exerted by the developing digestive tube. At about the fourth instar, the "mycetom" becomes

reduced to isolated groups of two, three, or more "mycetocytes," as a result of compression by the overcrowded and continually growing embryos. The organ is supplied with large tracheoles.

After differentiation of the definitive "mycetocytes," these cells divide at least once during the embryonic stages of the aphid, increasing in number from about thirty to forty to about sixty to seventy. After birth of the aphid, the "mycetocytes" apparently cease to undergo cell division. They merely increase in size, from about 42 to about 45 micra in the first instar to about 72 to 108 micra in the adult. The increase in size is evidently due, not only to the increase in number of "symbionts" inclosed in the cytoplasm, but also to growth of the cell itself. Upon reaching the adult stage, the "mycetocytes" begin to degenerate, one by one, until at about the close of the life of the aphid only very few are left in her abdominal cavity.

The "symbionts" never invade the nucleus of the "mycetocyte." The characteristic green color of the "mycetom" is apparently due to the green granular inclusions of the "microorganisms."

The "mycetom" is present in every individual aphid, whether amphigonous, male or female, or parthenogenetic. In parthenogenetic forms, the direct association of the host with the "symbionts" begins to take place during very early cleavage stages of the egg.

The germarium of the parthenogenetic aphid, on definitive differentiation, contains a number of cells, which are apparently all secondary, or ultimate oögonia. The latter apparently arise directly from the primitive germ cells. These cellular inclusions are far in excess of the total number of those which develop as ovarian eggs. The supernumerary oögonia apparently represent a reserve supply which insures the production of the maximum possible number of young. While in the germarium, these cellular inclusions probably have a secretory function, contributing to the nourishment of the young ovarian egg. The oögonia begin to develop into oöcytes at the posterior part of the germarium, and they are extruded in succession from this region into the vitellarium.

Extrusion of oöcytes into the vitellarium begins at about one day and fourteen hours to about three days and one hour, the average being about two days and seven hours, prior to birth of the mother. Two ovarian eggs are extruded into each vitellarium during the prenatal stages of the mother. Maturation

and subsequent cleavage of the egg take place soon after extrusion from the germarium. The time required for the development of the full-grown embryo, beginning with the extrusion of the oöcyte, covers a period of from about twelve days to about thirteen days and one hour, the average being about twelve days and thirteen hours. The entire embryonic life is passed in utero; and, in the case of about 75 per cent of the total number of young produced by each individual female, more or less advanced developmental stages are reached during the immature life of the mother. In as much as the extrusion and subsequent intrauterine development of the parthenogenetic aphid egg correspond to the deposition and subsequent incubation of the zygogenetic egg, ovulation and the subsequent development which take place during the preadult stages of the viviparous aphid represent a true case of pædogenesis, although the resulting young is born after the mother has reached the adult stage. A much larger percentage of parthenogenetic aphid young, therefore, is produced pædogenetically than otherwise. A redefinition of von Baer's original term is proposed in the present paper, wherein this method of reproduction, peculiar to aphids, is included under the subdivision of unisexual entopædogenesis.

The development of the parthenogenetic egg of aphids is described. The centrolecithal egg, which is familiar to all workers on arthropod embryology, differs in the case of the aphids from those of related groups in that the periplasm and, subsequently, the blastoderm leave an opening at the posterior pole. A direct communication between the follicular epithelium adjoining this region and the egg cavity is thereby established. This arrangement is apparently a special adaptation, accompanying "symbiosis," by which the "microörganisms" are enabled readily to enter the egg cavity.

Invagination occurs at the posterior opening of the blastoderm (blastopore). A portion of the "germ cylinder" thus formed thickens longitudinally and gives rise to the ventral plate; a corresponding thickening in the contiguous parietal area in the blastoderm gives rise to the procephalic lobes. The amnion and the serosa arise from the thinner parts of the "germ cylinder" and of the blastoderm, respectively. The former becomes considerably augmented by an extensible cellular sheet which arises from a secondary, posteriorly directed invagination at the anterior end of the "germ cylinder," the overgrowing layer ultimately closing the opening at that region. With the subsequent

growth of the ventral plate, this sheet is stretched by being pulled anteriorly by the former and finally becomes continuous posteriorly with the amnion.

The embryo at first grows with the head directed posteriorly. Blastokinesis occurs when the rudiments of the appendages and of the internal organs are well differentiated. The junction of the stomodæum with the proctodæum does not take place until after orientation of the embryo.

The mass of primitive germ cells becomes easily distinguishable when the "germ cylinder" is well formed, perhaps simultaneously with the early thickening of the ventral plate and the procephalic lobes. The mass of germ cells is constantly associated with the posterodorsal end of the ventral plate.

The formative egg follicle is represented by a large mass of cells adjoining the posterior portion of the terminal chamber, the former being evident from the time of the early differentiation of the latter. The egg follicle is formed by anterior circumcrescence of the formative egg follicle around the ovarian egg, until the latter is finally inclosed. At the completion of the process, the layer of epithelial cells covering the anterior pole of the egg proliferates into a thick, multilaminar mass. The latter structure takes the place of the original matrix of the egg follicle, and the process of formation of the eipithelum around the new ovarian egg is repeated, as described above.

The egg follicle apparently persists, without degenerating, through the latest embryonic stages. It probably constitutes the thin, membranous envelope which covers the young at parturition. This usually ruptures before extrusion of the young through the vaginal slit is completed.

The parthenogenetic *Macrosiphum* has twelve ovarioles, arranged bilaterally in two equinumeral groups. They are attached anteriorly, by means of terminal filaments, to the dorsal diaphragm, at about the junction of the metathoracic and the first abdominal segments. Posteriorly, each of the groups of ovarioles joins one of the two short oviducts. The vagina is characterized by a relatively much thinner wall than that of the amphigonous aphid, and by the absence of spermatheca and of colleterial glands.

A review of the literature on reproduction and parthenogenetic embryology, and also on "symbiosis" and "symbiotic organs" in aphids is given in the present paper.

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ILLUSTRATIONS

[All figures represent parthenogenetic material, unless otherwise specified. Magnifications indicated refer to size of original figures.]

ABBREVIATIONS

ad, adipose tissue.
am, amnion.
an, anus.
ap, anal plate.
at, antennæ.
b, small anterior nuclei.
bl, blastoderm.
br, brain.
c, vestige of matrix of egg follicle.
caud, abdominal cauda.
ce, cerebral lobes.
crn, cornicle.
cyl, "germ cylinder."
d, thickened anterior portion of blastoderm and, later, of serosa.
e, portion of secondary invagination of "germ cylinder" over amnion.
em, entoderm.
eo, embryo.
f, portion of secondary invagination of "germ cylinder" over ventral plate.
fb, formative blastoderm.
fe, egg follicle.
fil, terminal filament.
fm, formative mycetom.
g, indusium (?).
gc, germ cells.
gm, germarium, or terminal chamber.
gon, rudimentary gonapophysis.
gp, genital plate.
hg, hind gut.
hyp, hypodermis.
im, investing membrane of "mycetom."
int, chitinous intima.
lv, labium.
mb, "mycetoblast" ("vitellophag").
md, mandible.
mem, basement membrane.
mi, "mid-intestine" (bulb-shaped thickening of foregut).
mp, maxillary palpus.
ms, mesentoderm.
mu, muscle.
mx, maxilla.
my, "mycetom."
oc, oöcyte.
oe, œnocyte.
oes, œsophagus.
og, oögonium.
op, vaginal slit.
or, ovary.
ov, ovarian egg.
ovd, oviduct.
pc, procephalic lobes.
pe, anlage of leg.
pm, periplasm.
pr, proctodæum.
se, serosa.
sg, subœsophageal ganglion.
sm, stomodæum.
sv, salivary gland.
ta, thoracic ganglia.
tr, trachea.
vag, vagina.
vit, vitellarium.
vlv, anterior valve of "mid-gut."
vp, ventral plate.

PLATE 1

FIG. 1. *Macrosiphum tanaceti;* apterous adult, caudal portion of abdomen, lateral view. × 175.

2. *Drepanosiphum platanoides;* nymph, sagittal section through right side, showing position of "mycetom" in abdominal cavity, 7 micra; Gilson's fixative; Ehrich's hæmatoxylin. × 175.

FIGS. 3–9. *Myzus persicæ;* successive stages of embryos. Figs. 3 and 4, latest stages prior to blastokinesis; fig. 5, immediately after blastokinesis; figs. 6, 7, 8, and 9, older embryos, showing subsequent development of appendages. Bouin's fixative; Gage's Säurefuchsin. × 365.

FIG. 10. *Macrosiphum tanaceti;* full-grown embryo, about ready to be born, ventral view, fresh; Ringer's solution. × 175.

11. *Macrosiphum tanaceti;* same age as fig. 10, sagittal section, 5 micra; Webster and Phillips's fixative; Ehrlich. × 365.

PLATE 2

FIG. 12. *Macrosiphum rosæ;* central nervous system of full-grown embryo, sagittal section, 7 micra; Gilson; Ehrlich. × 365.

13. *Macrosiphum tanaceti;* longitudinal section through "mid-intestine" of embryo, showing development of anterior valve, 7 micra; Gilson; Ehrlich. × 720.

14. *Macrosiphum rosæ;* longitudinal section through anterior portion of "mid-intestine" of adult, 7 micra; Gilson; Ehrlich. × 720.

15. *Macrosiphum tanaceti;* longitudinal section through spiracle and contiguous portion of trachea, 7 micra; Kleinenberg's fixative; Ehrlich. × 1,400.

16. *Macrosiphum rosæ;* adult; sagittal section through caudal portion of abdomen, showing vagina and portion of one of the ovaries, 7 micra; Gilson; Ehrlich. × 700.

17. *Macrosiphum tanaceti;* longitudinal section through portion of one of the oviducts and of a contiguous vitellarium, showing persisting egg follicle around a full-grown embryo, 6 micra; Kleinenberg; Ehrlich. × 1,400.

18. *Macrosiphum tanaceti;* left two-thirds of cross section of vagina, 6 micra; Kleinenberg; Ehrlich. × 1,400.

PLATE 3

FIG. 19. *Macrosiphum tanaceti;* mass of germ cells in embryo before metameric segmentation of latter, 7 micra; Gilson; Ehrlich. × 2,800.

20. *Macrosiphum rosæ;* ovariole of very young embryo; cellular inclusions of germarium all oögonia; longitudinal section, 7 micra; Gilson; Ehrlich. × 1,400.

21. *Macrosiphum rosæ;* ovariole of older embryo, showing passing of ultimate oögonia into oöcytes, preparatory to extrusion of latter, longitudinal section, Gilson; Ehrlich. × 1,400.

FIG. 22. *Macrosiphum tanaceti;* anterior portion of ovariole of nymph, first instar, showing extrusion of "ovarian egg" and formation of egg follicle, longitudinal section, 5 micra; Gilson; Ehrlich. × 1,400.

23. *Macrosiphum tanaceti;* anterior portion of ovariole of nymph, second instar, showing completion of egg follicle around "ovarian egg," longitudinal section, 7 micra; Gilson; Ehrlich. × 1,400.

24. *Macrosiphum tanaceti;* oögonium, 7 micra; Gilson; Ehrlich, × 1,400.

25. *Macrosiphum tanaceti;* full-grown "ovarian egg," prior to extrusion of polar body, longitudinal section, 6 micra; Gilson; Heidenhain-eosin. × 1,400.

26. *Macrosiphum tanaceti;* egg, extrusion of polar body, longitudinal section, 6 micra; Gilson; Heidenhain-eosin. × 1,400.

27. *Macrosiphum tanaceti;* egg, after extrusion of polar body; one-nucleus stage, longitudinal section, 7 micra; Gilson; Ehrlich. × 1,400.

PLATE 4

FIGS. 28–32. *Macrosiphum tanaceti;* eggs, cleavage stages, longitudinal section, 7 micra; Gilson; Ehrlich. × 1,400.

33–35. *Macrosiphum tanaceti;* eggs, preliminary steps in blastoderm formation, longitudinal section, 7 micra; Gilson; Ehrlich. × 1,400.

PLATE 5

FIG. 36. *Macrosiphum tanaceti;* egg, late preblastodermal stage, longitudinal section, 7 micra; Gilson; Ehrlich. × 1,400.

37. *Macrosiphum tanaceti;* egg, differentiation of blastomeres, longitudinal section, 7 micra; Gilson; Ehrlich. × 1,400.

38. *Macrosiphum tanaceti;* egg, early blastoderm stage, longitudinal section, 7 micra; Gilson; Ehrlich. × 1,400.

39. *Macrosiphum tanaceti;* egg, blastoderm stage, immediately before invagination of "germ cylinder," longitudinal section, 7 micra; Gilson; Ehrlich. × 1,400.

PLATE 6

FIG. 40. *Macrosiphum tanaceti;* egg, blastoderm stage; invagination of "germ cylinder," longitudinal section, 7 micra; Gilson; Delafield. × 1,400.

41. *Macrosiphum tanaceti;* egg, differentiation of ventral plate and procephalic lobes, segregation of germ cells, longitudinal section, 5 micra; Gilson; Ehrlich. × 1,400.

42. *Macrosiphum tanaceti;* section through "mycetocyte" of full-grown embryo, 5 micra; Webster and Phillips; Ehrlich. × 1,400.

43. *Macrosiphum tanaceti;* section through "mycetocyte" of adult, 5 micra; Webster and Phillips; Ehrlich. × 1,400.

PLATE 7

FIG. 44. *Macrosiphum tanaceti;* egg, beginning of metameric segmentation of germ band, sagittal section, 7 micra; Kleinenberg; Heidenhain-eosin. × 720.

45. *Macrosiphum tanaceti;* egg, prior to metameric segmentation of germ band, before cellular differentiation of "mycetocytes." Note the persisting swollen part of egg follicle adjoining the posterior pole. Sagittal section, 7 micra; Gilson; Ehrlich. × 1,400.

46. *Macrosiphum rosæ;* embryo, nearly full grown, sagittal section, 7 micra; Gilson; Ehrlich. × 1,400.

PLATE 8

FIG. 47. *Macrosiphum tanaceti;* formative "mycetom," with "mycetoblasts" in mitosis, preparatory to differentiation of "mycetocytes," 7 micra; Gilson; Ehrlich. × 1,400.

48. *Macrosiphum tanaceti;* egg, slightly older than fig. 45, shortly before metameric segmentation of germ band, definitive "mycetocytes" differentiated, sagittal section, 5 micra; Gilson; Ehrlich. × 1,400.

49. Unidentified species on *Lythrum;* egg, metameric segmentation of germ band, sagittal section, 7 micra; Gilson; Heidenhain-eosin. × 720.

PLATE 9

FIG. 50. *Macrosiphum tanaceti;* portion of "mycetom," showing tracheation and investing membrane, 7 micra; Kleinenberg; Ehrlich. × 1,400.

51. *Macrosiphum tanaceti;* embryo, shortly before blastokinesis, sagittal section, 5 micra; Kleinenberg; Ehrlich. × 1,400.

PLATE 10

FIG. 52. Unidentified species on *Lythrum;* embryo, showing development of appendages and of internal organs, prior to blastokinesis, sagittal section, 7 micra; Gilson; Heidenhain-eosin. × 1,400.

53. *Macrosiphum tanaceti;* embryo, slightly older than stage corresponding to fig. 52, prior to blastokinesis, sagittal section, 6 micra; Kleinenberg; Heidenhain-eosin. × 720.

54. *Macrosiphum tanaceti;* details of one of the developing thoracic legs of fig. 53. × 1,400.

PLATE 11

FIG. 55. *Macrosiphum tanaceti;* young embryo, about the same age as fig. 56, cross section, through anterior portion of abdomen, showing preliminary steps in bipartition of "mycetom," 5 micra; Gilson; Ehrlich. × 720.

56. *Macrosiphum tanaceti;* embryo, shortly after blastokinesis, sagittal section, 5 micra; Webster and Phillips; Ehrlich. × 720.

PLATE 12

FIG. 57. *Macrosiphum tanaceti;* embryo, the same age as fig. 56, sagittal section through caudal portion of abdomen, showing early relation of proctodæum with "mycetom," 5 micra; Webster and Phillips; Ehrlich. × 1,400.

PLATE 13

FIGS. 58–62. *Macrosiphum rosæ;* embryo, nearly full grown, cephalocaudal series of cross sections; figs. 58 and 59, through head; figs. 60 and 61, through thorax; fig. 62, through subcaudal portion of abdomen; 7 micra; Gilson; Ehrlich. × 365.

FIG. 63. *Macrosiphum rosæ;* embryo, the same age as the preceding; frontal section through central nervous system, 7 micra; Gilson; Ehrlich. × 365.

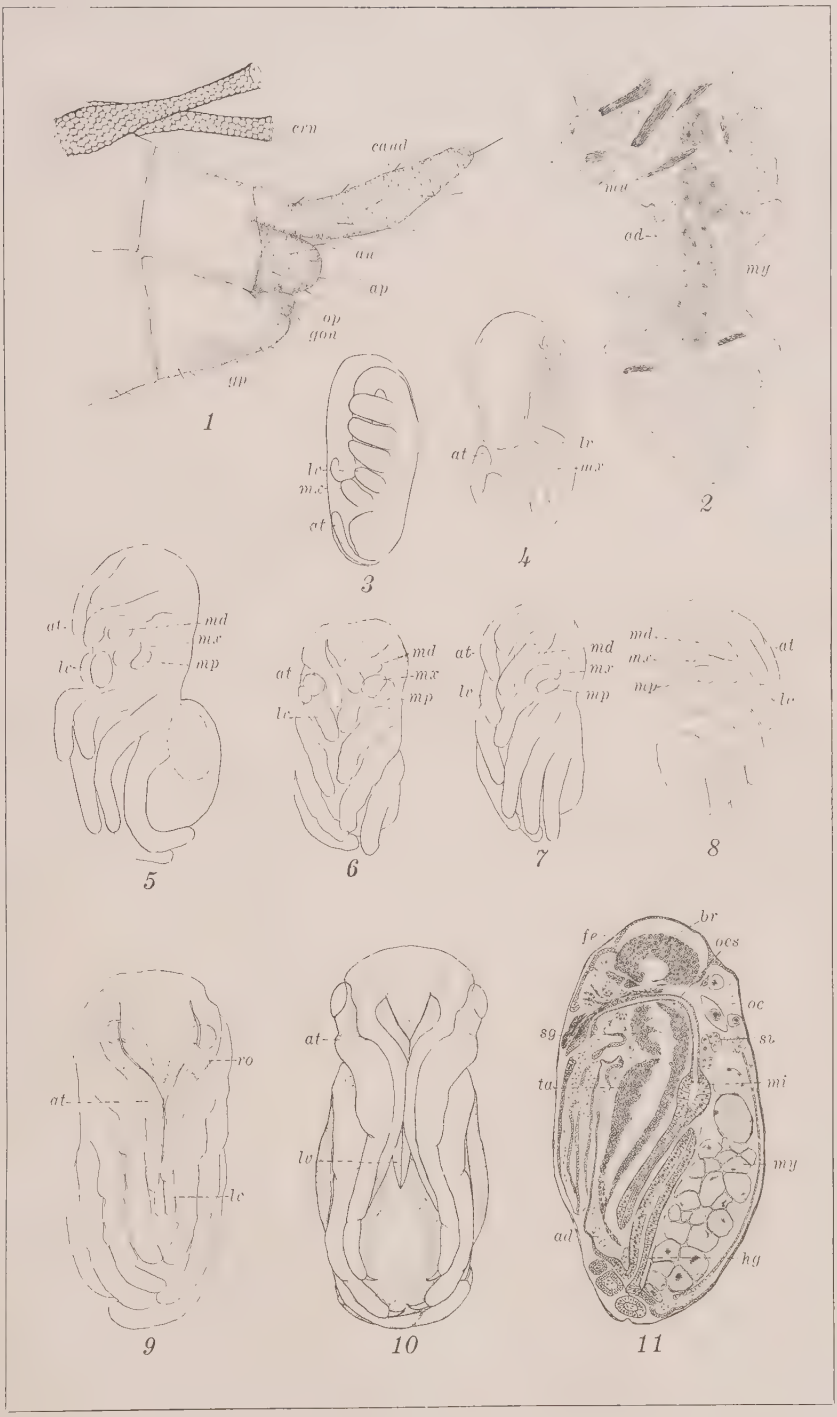
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PLATE 1.

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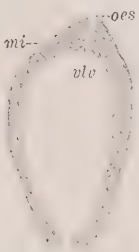

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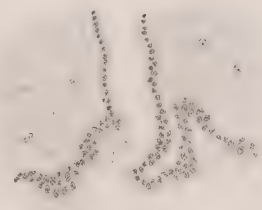

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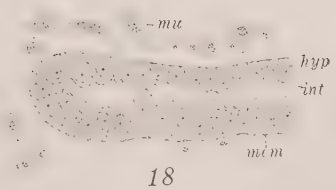

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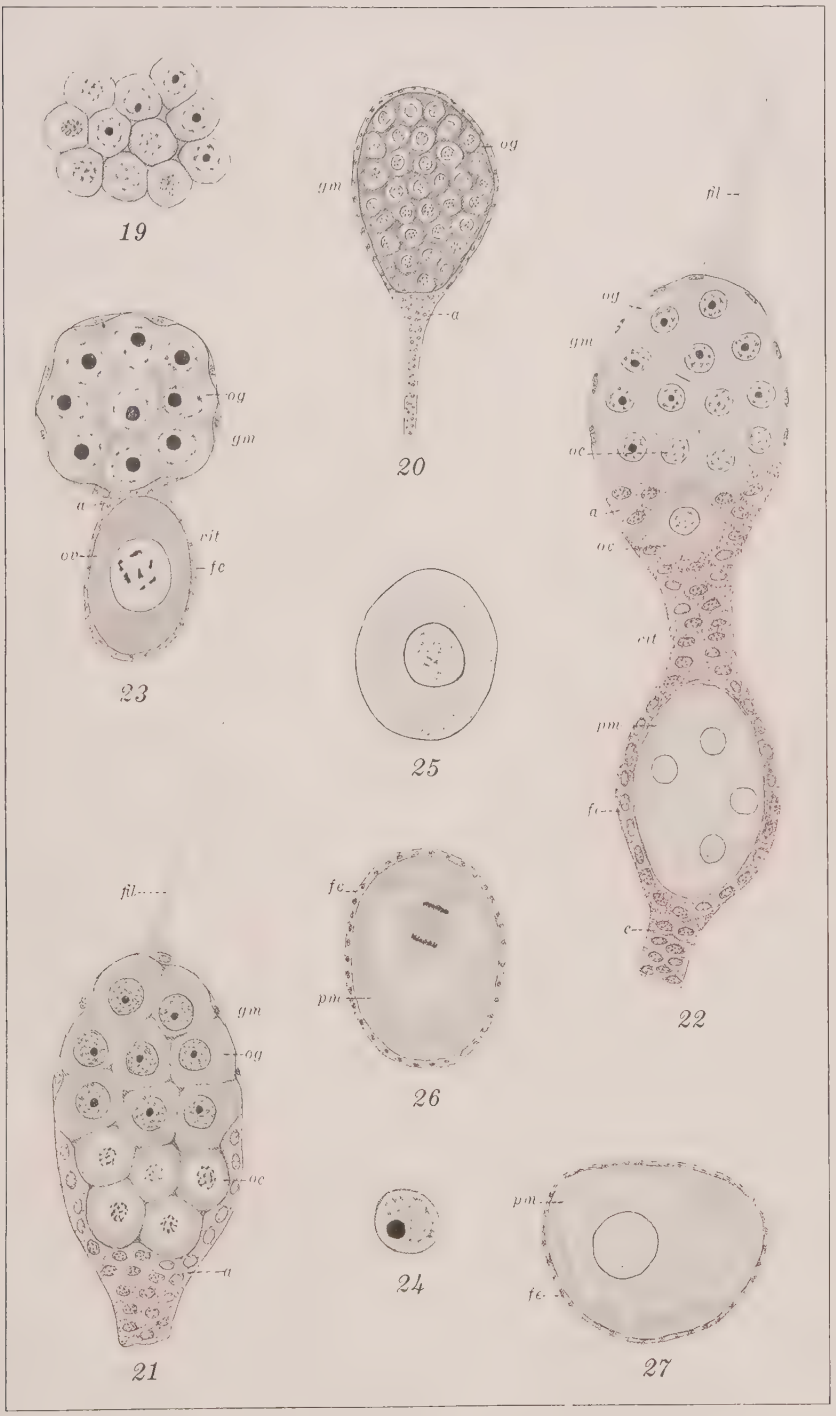
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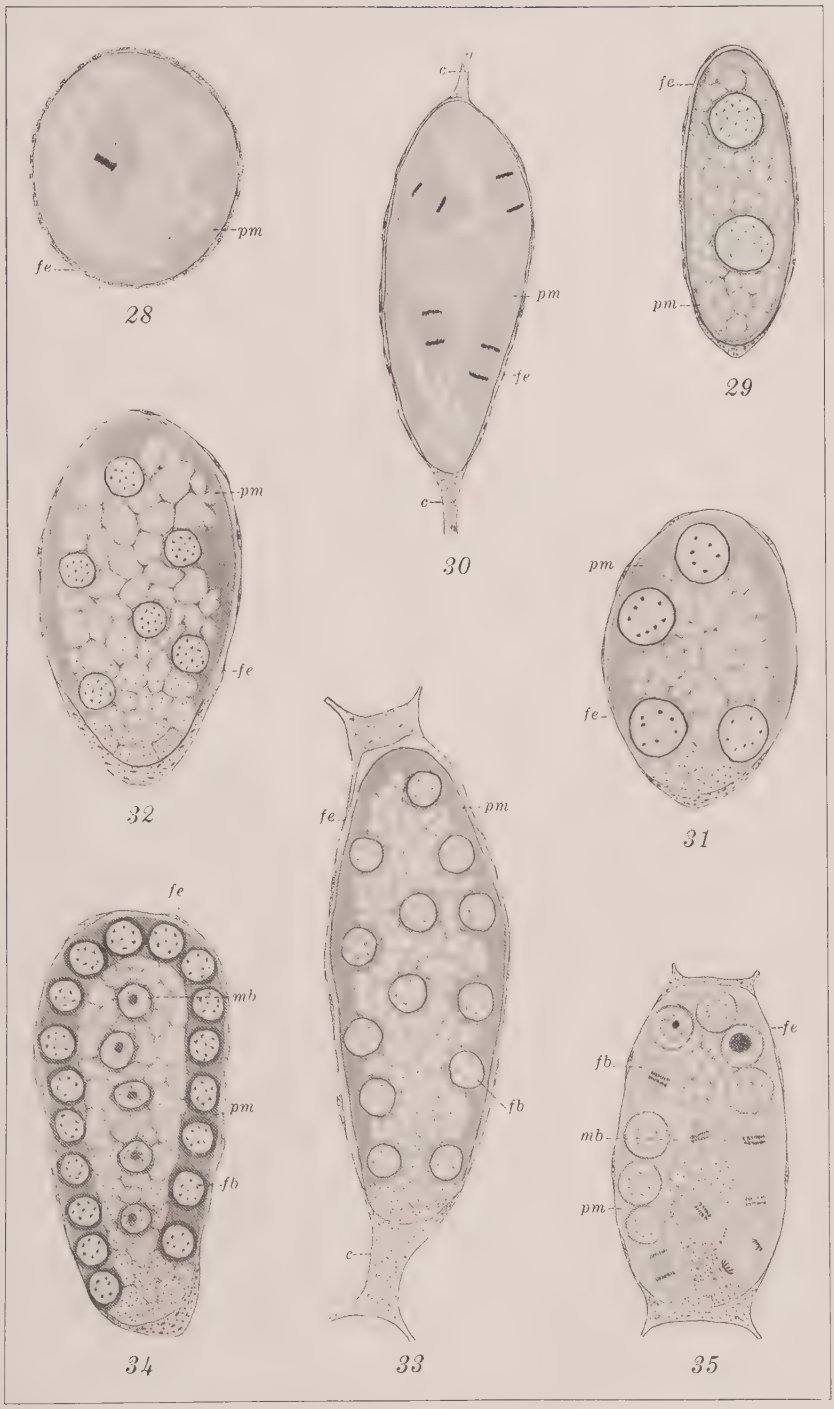
PLATE 3.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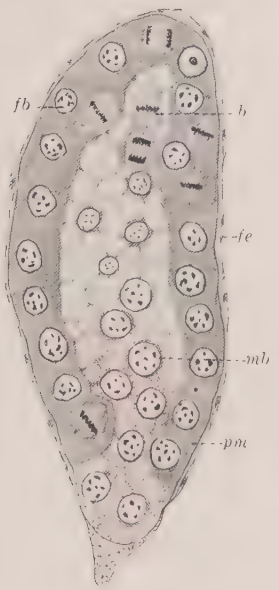

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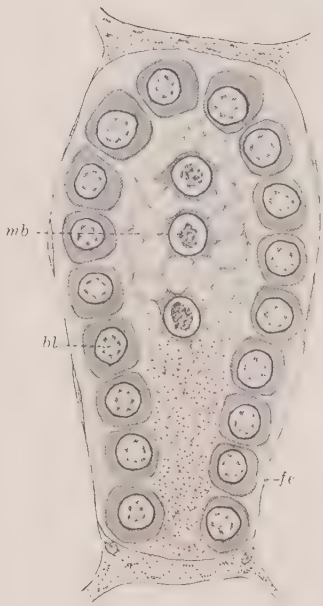

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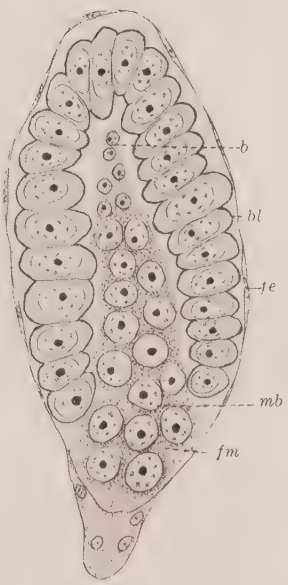

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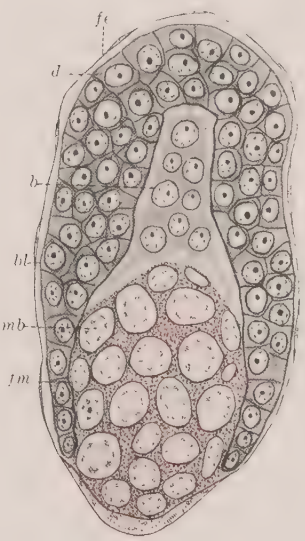

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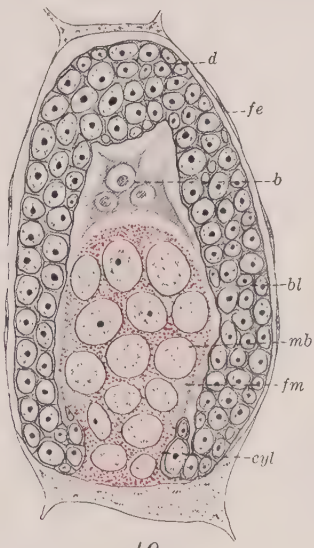

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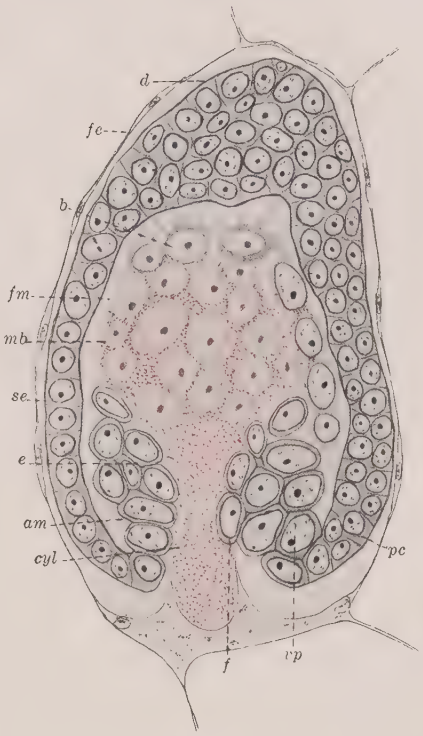

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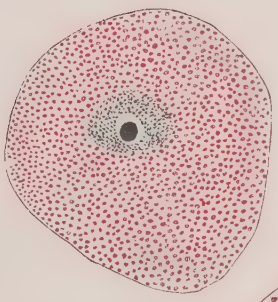

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PLATE 6.

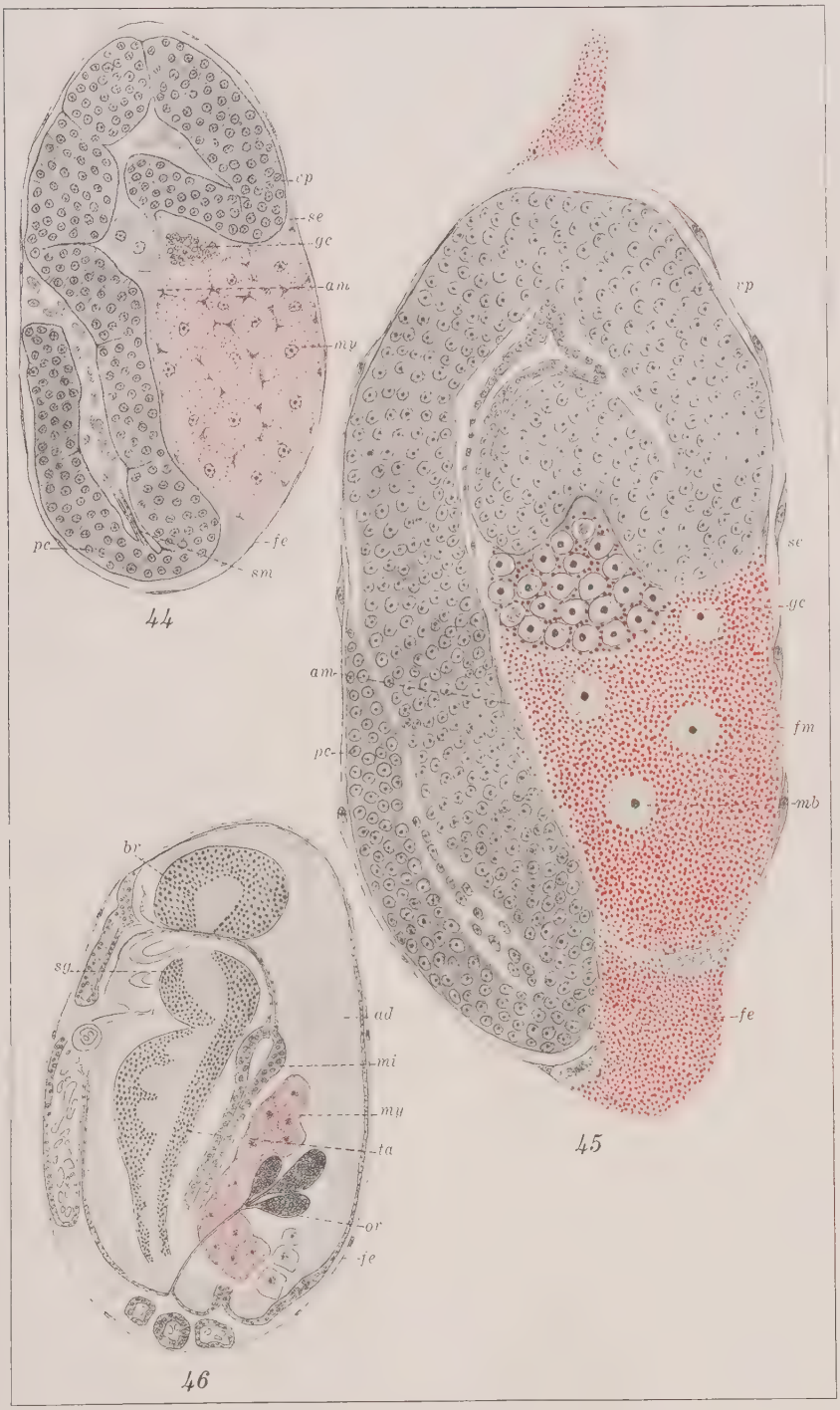
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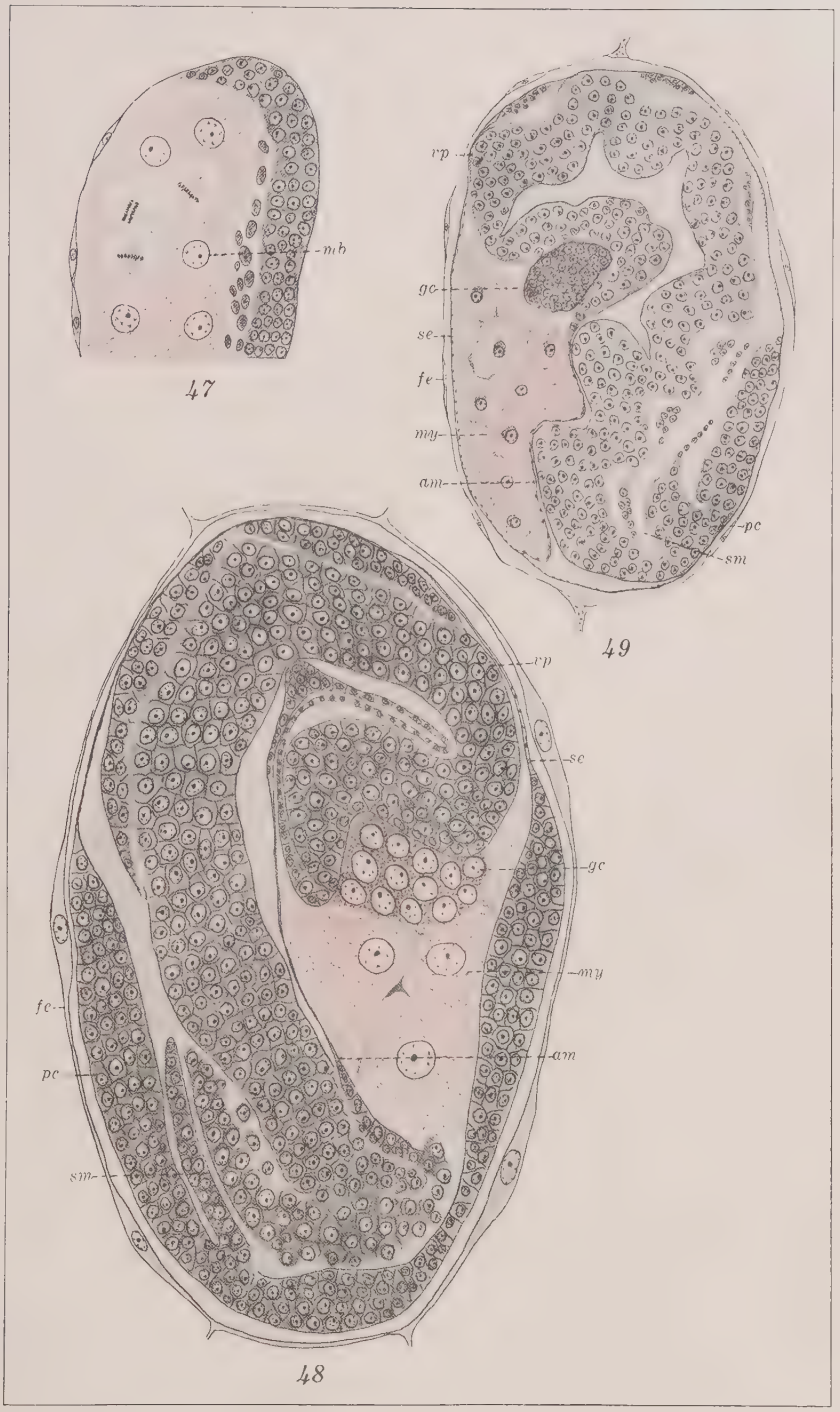
PLATE 7.



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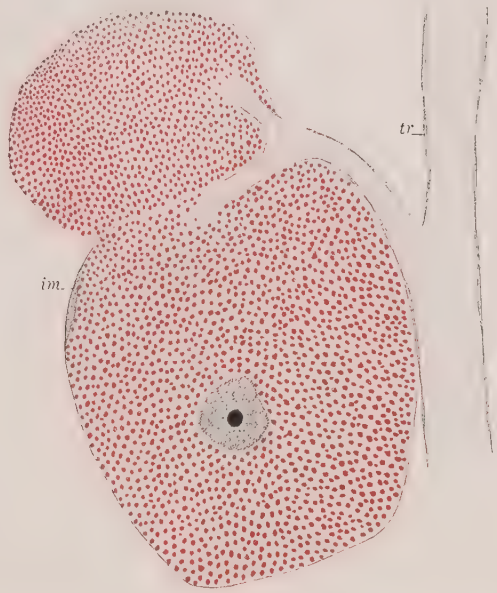

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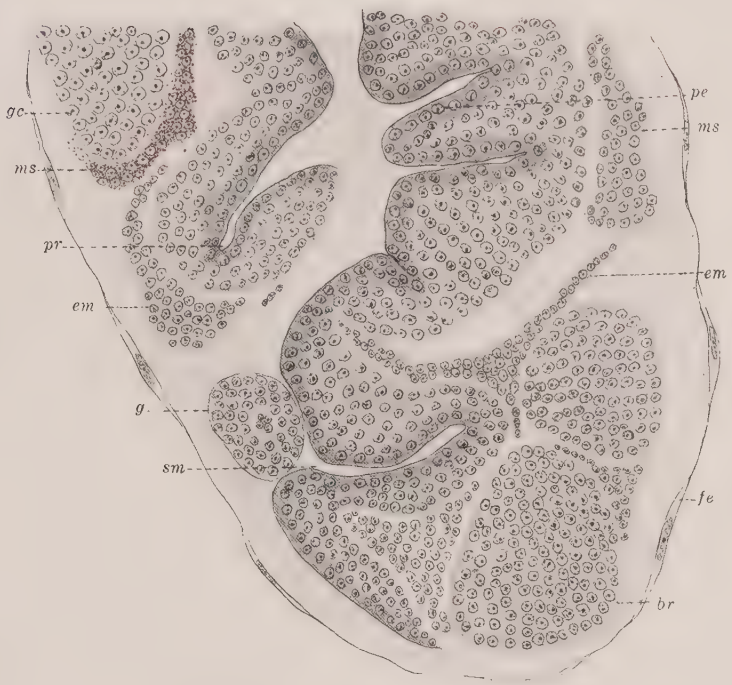

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PLATE 9.

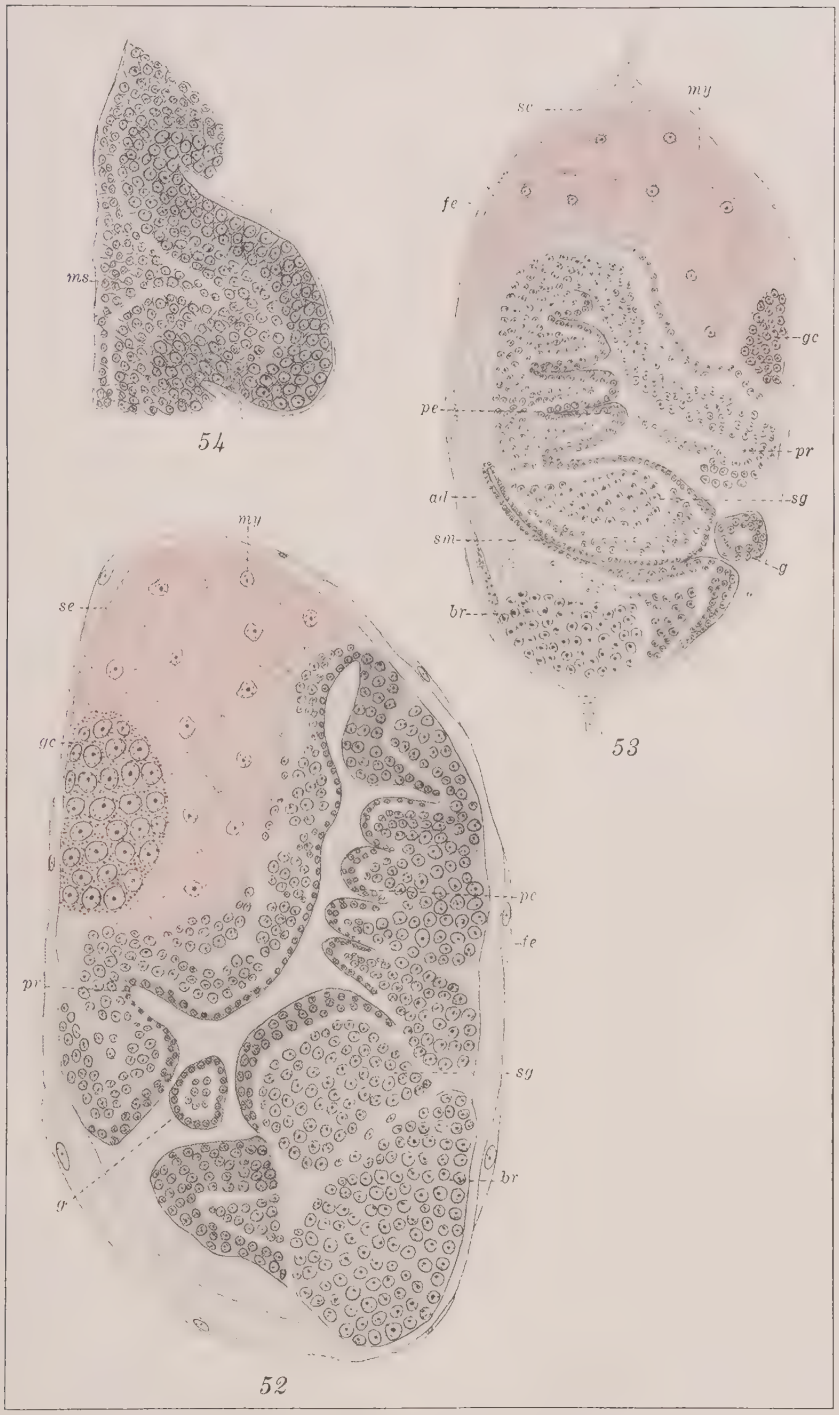
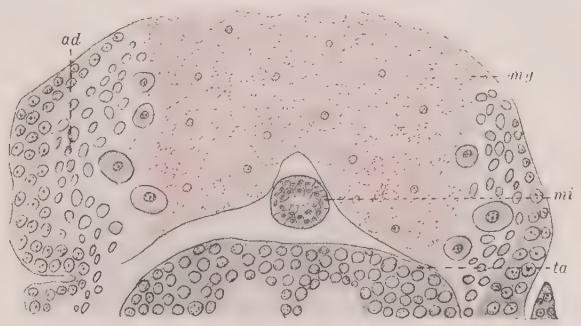


PLATE 10.



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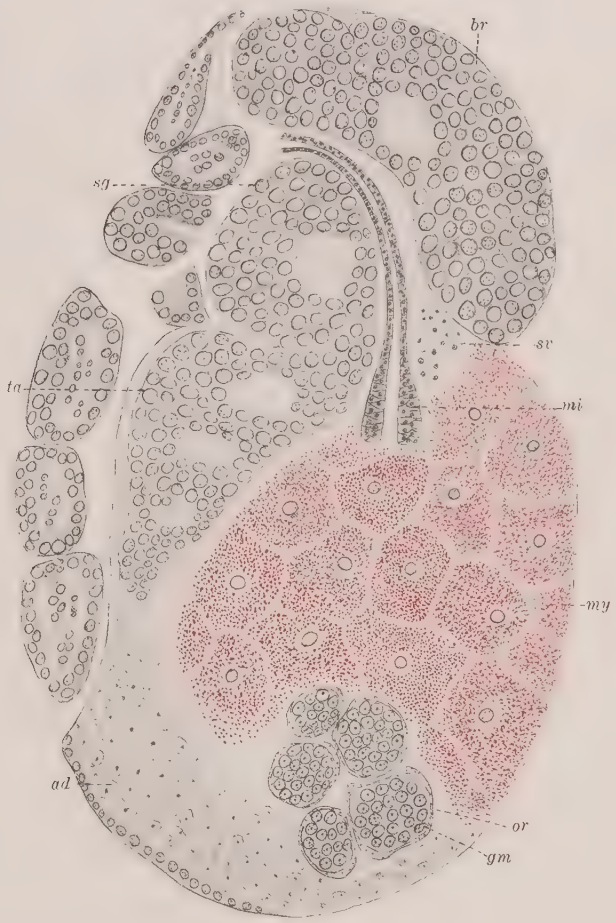

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PLATE 11

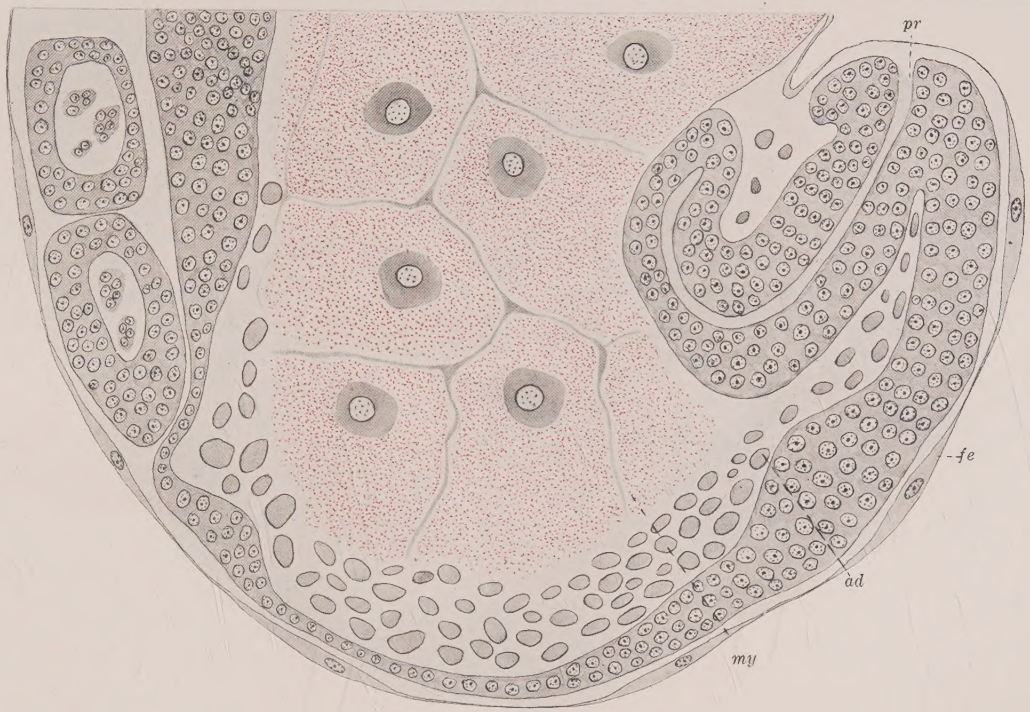

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PLATE 12.

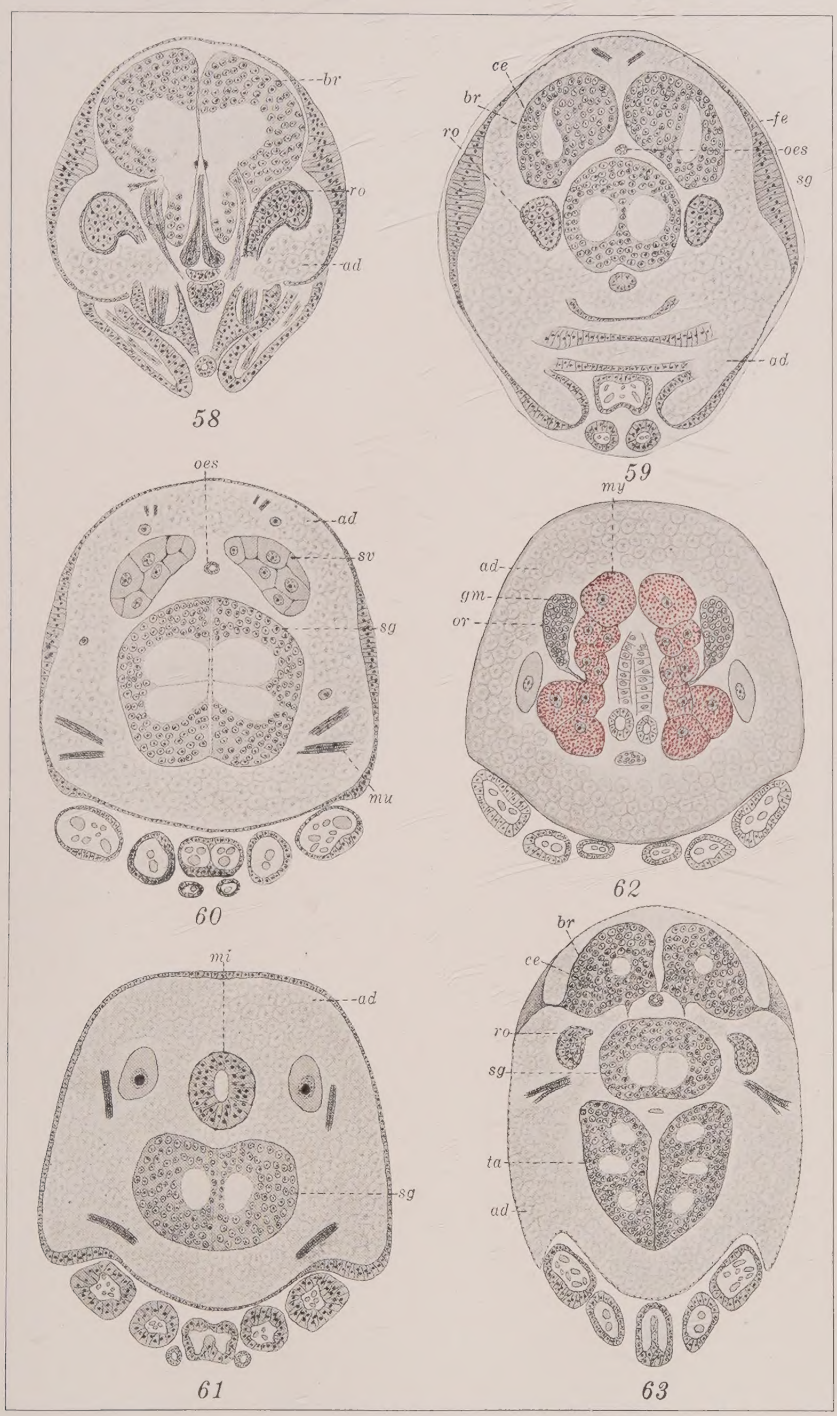
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PLATE 13.